ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Finding the High Specific Target Gene Among Cry1Ac & Cry2Ab In Resistance To Pesticide.

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ABSTRACT

Bacillus *thuringiensis*(Bt) is a very important organism in the present day biological experiments. It is a soil bacterium and now-a-days it is also being used to transfer some required genes which can improve the resistance of certain plants by killing larvae like Lepidoptera especially, Spodoptera insects. Cry (Crystal genes) genes are the important genes in the Bt which can improve resistance in the plants. Among the Cry genes, Cry1Ac & Cry2Ab genes are the important resistance giving genes. These should be transferred to the plants because Spodoptera larva are the infectious ones which cause drastic damage to the plants and rapidly decreases the productivity by the infectious proteins in them. Homology modeling is a technique in which we can design the structure of required proteins like 6-aminobutarate aminotransferase. The structure of Cry1Ac & Cry2Ab proteins are modeled using various Bio-Informatics tools. Then by docking these proteins with the disease causing protein of the Spodoptera larva and comparing the energies of the best minimized structures, we can conclude which Crysal protein suits best to control these larvae and gives more resistance. So that we can check for the presence of that particular protein in the hybrid crops and increase their productivity.

Keywords:6-aminobutarate aminotransferase,BT, Homology modeling

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ISSN: 0975-8585

COTTON" is a soft, fluffy staple fiber that grows in a boll, or protective capsule, around the seeds of cotton plants of the genus **Gossypium**. The plant is a shrub native to tropical and subtropical regions around the world, including the America, Africa, India, and Pakistan. The fiber most often is spun into yarn or thread and used to make a soft, breathable textile, which is the most widely used natural-fiber cloth in clothing today.

Scientific classification of Gossypium*hirsutum*

KINGDOM	**:**	Plantae
SUBKINGDOM	**:**	Tracheobionta
SUPERDIVISION	**:**	Spermatophyta
DIVISION	**:**	Magnoliphyta
CLASS	**:**	Magnoliopsida
SUBCLASS	**:**	Dillendilae
ORDER	**:**	Malvales
FAMILY	**:**	Malvaceae
GENUS	**:**	Gossypium
SPECIES	**:**	*hirsutum*

But now-a-days, cotton crop is being susceptible to many diseases/infections caused by so many pests and insects. These all should be controlled inorder to protect and increase the productivity of the crop. These infections should be studied to find a solution to this problem and increase the productivity.

SPODOPTERA:

SCIENTIFIC CLASSIFICATION:

SUBCLASS	:	Pterygota
INFRACLASS	:	Neoptera
SUPERORDER	:	Endopterygota
ORDER	:	Lepidoptera
GENUS	:	Spodoptera
SPECIES	:	*littoralis*

(LARVAL FORM)

(ADULT FORM)

There are many species of Spodoptera which causes damage to the crops**.Spodoptera** is a genus of moths of the Noctuidae family. Many are known as pest insects. The larvae are sometimes called armyworms. Species include:

DAMAGE

The caterpillars of this moth feed mainly on leaves, munching on their edges and making holes, giving them a ragged and torn appearance.If the larvae are numerous, they can completely defoliate the plants. When they have exhausted their food source, they invade neighbouring vegetation - which may often be another crop - in large numbers ("armyworm"), devouring almost all suitable plant material in their path. Infestations often remain unnoticed at first, as the small larvae prefer feeding sites close to the ground. By far

most of the damage, however, is caused by the last instar larvae, which consume more plant biomass than all of the other instars put together. This often makes attacks by fall armyworm seem very sudden.

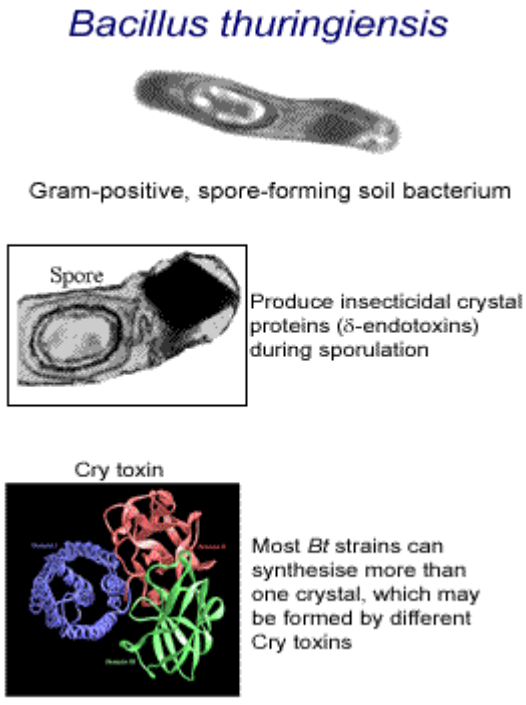
There are some preventive measures to control these pests like some pesticides, but they are not effective. So, some other alternatives should be searched. The genome of the Cotton plant can be altered. An organism named Bacillus *thuringiensis* have some genes which can give more resistance namely, Crystal proteins. So, it is very much important to modify the crop's genome and produce genetically modified(GM) crops [1]. This procedure gives us more resistive crop and also more productivity. Inorder to apply this procedure, first we need to study about the genome of the Bacillus *thuringiensis* and then about the gene transfer using vectors.

Bacillus *thuringiensis* (or Bt):

Bacillus *thuringiensis* is a Gram-positive, soil-dwelling bacterium, commonly used as a biological alternative to a pesticide; alternatively, the Cry toxin may be extracted and used as a pesticide. *B. thuringiensis* also occurs naturally in the gut of caterpillars of various types of moths and butterflies, as well as on the dark surface of plants. This has led to their use as insecticides, and more recently to genetically modified crops using Bt genes. There are however many crystal-producing Bt strains that do not have insecticidal properties.

Genome description of Bacillus *thuringiensis*:

The genome of Bacillus thuringiensis consists of nearly 500 genes. These genes are **CRYSTAL** (Cry) proteins. These genes are mainly divided into Cry1, 2, 3, 4, 5, 6, 7, 8, 9. then they are again divided into groups Cry1A, Cry1B,…, Cry2A, Cry2B,…,etc., Later they are again divided into Cry1Aa, Cry1Ab,…, Cry2Aa, Cry2Ab,…,etc., But among all these genes in the genome of the Bacillus thuringiensis, **Cry1Ac, Cry2Ab** are the genes which gives more resistance and can be transferred to the Gossypium genome for giving more resistance power. These Cry proteins are endotoxins and can be used to kill the pests of the cotton crop.



Cry toxins have specific activities against insect species of the orders Lepidoptera (moths and butterflies), Diptera (flies and mosquitoes), Coleoptera (beetles), hymenoptera (wasps, bees, ants and sawflies) and nematodes. Thus, *B. thuringiensis* serves as an important reservoir of Cry toxins for production of biological insecticides and insect-resistant genetically modified crops. When insects ingest toxin crystals, the alkaline pH of their digestive tract activates the toxin. Cry inserts into the insect gut cell membrane, forming a pore. The pore results cell lysis and eventual death of the insect.

ISSN: 0975-8585

Since cotton is a very important commercial plant, it's resistance over the pests and insects should be more. Among the pests infecting the Gossypium(cotton) crop,larvae of Lepidoptera and Spodoptera are the most infectious.

Inorder to increase the productivity of the cotton crop, we need to supress the afffect of these pests. This can be done by using endotoxins. These endotoxins can be produced by Bacillus *thuringiensis*(Bt). So, we have to transfer the genome of Bt into the cotton genome using various vectors.

USAGE OF VECTORS TO TRANSFER THE GENES

Essentially, gene transfer involves the delivery of therapeutic protein encoding nucleic acid to the target cells. The DNA consists of one or more genes to be expressed in the target cell and the sequences controlling their expression. The administration of genes as therapy requires transportation vehicles, called vectors, which encapsulate the gene and carry it into the target cell. The vector binds to the target cell membrane, and after this internalization takes place. The administered genome is then transported into the cell nucleus, where it is integrated into the genome of the cell or remains outside the genome as an episome in the nucleus, depending on the type of vector used. Every step in this process and the ultimate expression of the gene constitute potential limitations of gene therapy. If the 2-µm plasmid is used as the basic vector and other bacterial and yeast segments are spliced into it, then a construct having several useful properties is obtained [2]. First, the 2µm segment confers the ability to replicate autonomously in the yeast cell, and insertion is not necessary for a stable transformation. Second, genes can be introduced into yeast, and their effects can be studied in that organism; then the plasmid can be recovered and put back into *E. coli,* provided that a bacterial replication origin and a selectable bacte-rial marker are on the plasmid. Such **shuttle vectors** are very useful in the routine cloning and manipulation of yeast genes.

PROTEIN DATA BANK (PDB):

The Protein Data Bank (PDB) format provides a standard representation for macromolecular structure data derived from X-ray diffraction and NMR studies.

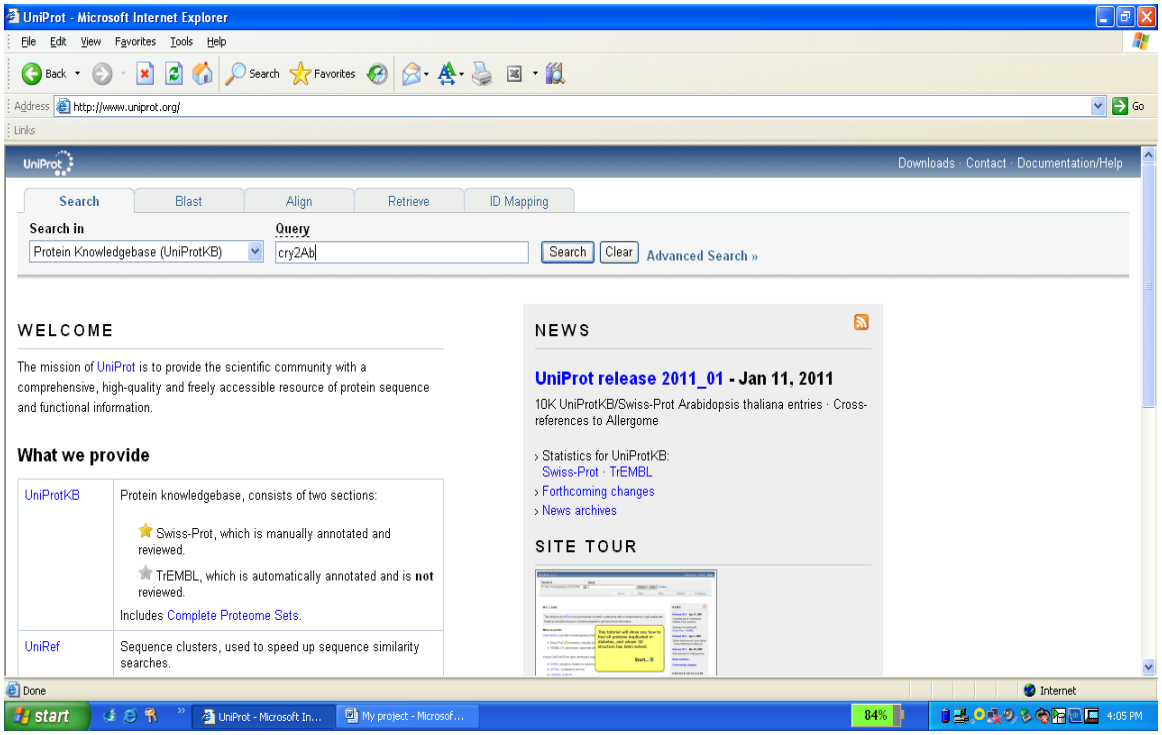
The file format initially used by the PDB was called the PDB file format. This original format was restricted by the width of computer punch cards to 80 characters per line. Around 1996, the "macromolecular Crystallographic Information file" format, mmCIF, started to be phased in. The "4hhb" is the PDB identifier. Each structure published in PDB receives a four-character alphanumeric identifier, its PDB ID. (This cannot be used as an identifier for biomolecules, because often several structures for the same molecule—in different environments or conformations—are contained in PDB with different PDB IDs.)The RCSB PDB also provides a

variety of tools and resources. These molecules are visualized, downloaded, and analyzed by users who range from students to specialized scientists [4].

UNIPROT

The UniProt Knowledgebase (UniProtKB) is the central hub for the collection of functional information on proteins, with accurate, consistent and rich annotation. In addition to capturing the core data mandatory for each UniProtKB entry (mainly, the amino acid sequence, protein name or description, taxonomic data and citation information), as much annotation information as possible is added. This includes widely accepted biological ontologies, classifications and cross-references, and clear indications of the quality of annotation in the form of evidence attribution of experimental and computational data.

The UniProt Knowledgebase consists of two sections: a section containing manually-annotated records with information extracted from literature and curator-evaluated computational analysis, and a section with computationally analyzed records that await full manual annotation.



SWISS MODEL

SWISS-MODEL is a fully automated protein structure homology-modeling server, accessible via the ExPASy web server, or from the program Deep View (Swiss Pdb-Viewer). The purpose of this server is to make Protein Modeling accessible to all biochemists and molecular biologists Worldwide. The SWISS-MODEL Repository is a database of annotated three-dimensional comparative protein structure models generated by the fully automated homology-modelling pipeline SWISS-MODEL.

SWISS-MODEL is developed by the Protein Structure Bioinformatics group at the SIB - Swiss Institute of Bioinformatics and the Biozentrum University of Basel

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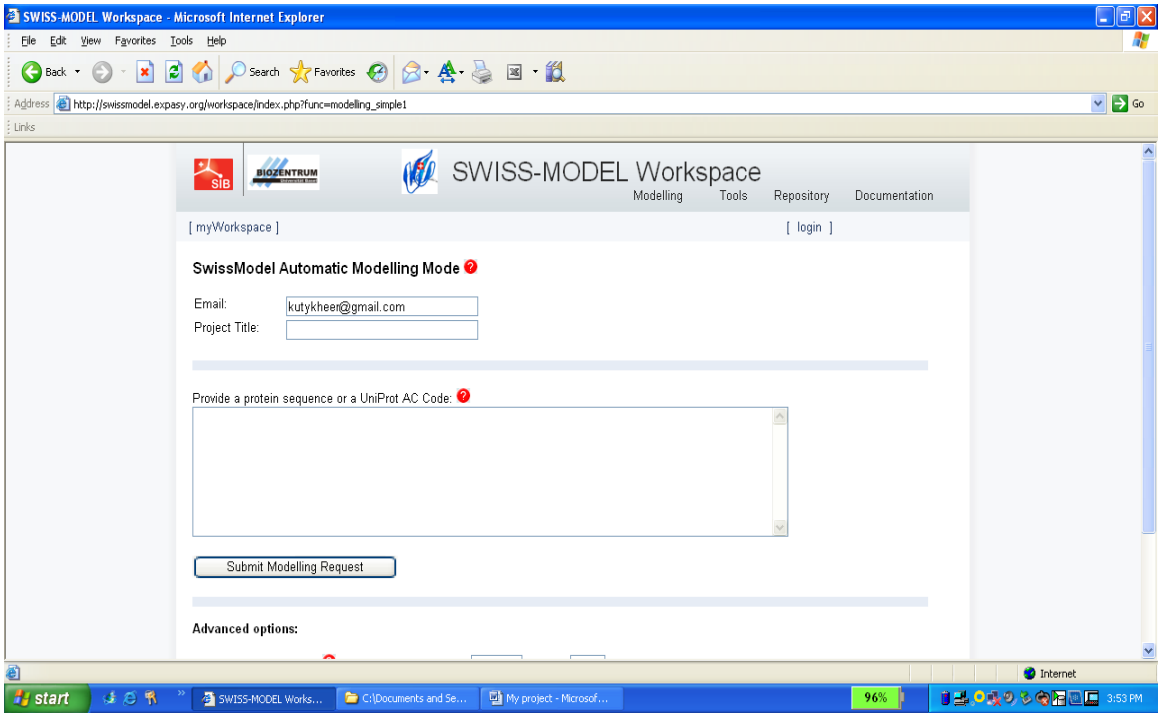
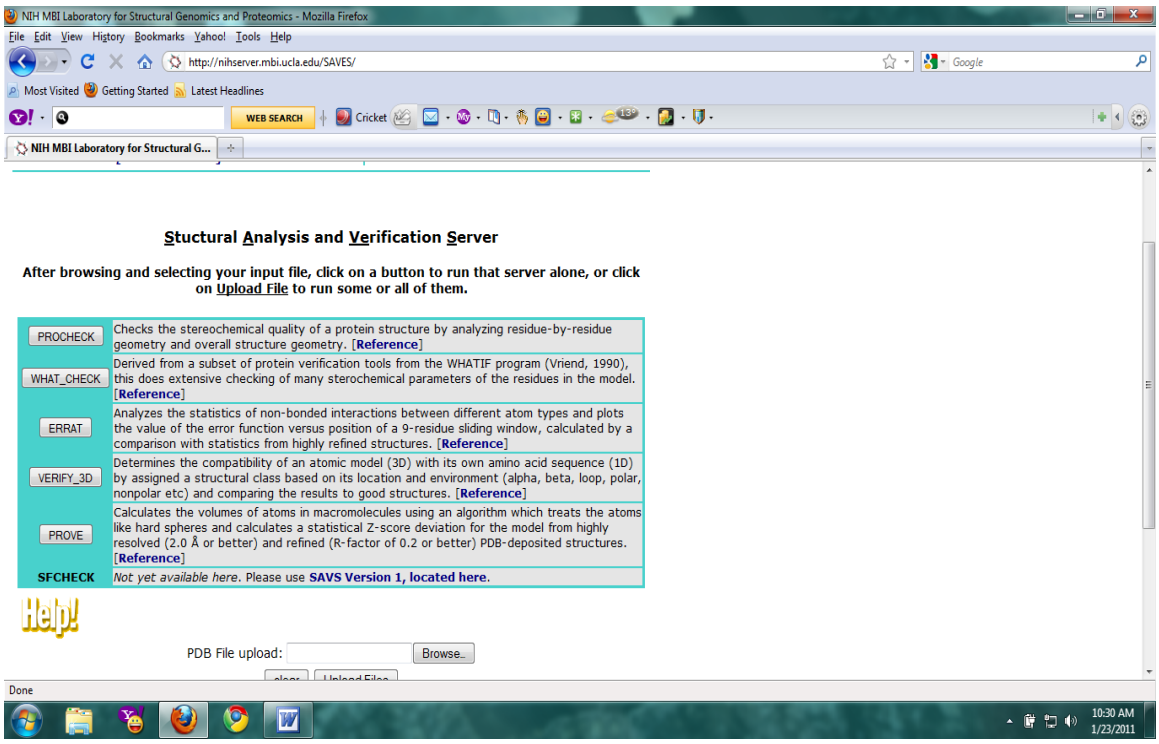


STRUCTURE ANALYSIS AND VERIFICATION SERVER (SAVS):

The below is the snapshot of the SAVS server which validates the protein structures:



SAVS is an online verification and validation server having some specific tests like PROCHECK, WHAT_CHECK, ERRAT, VERIFY_3D, PROVE. These are the tests / checks to verify whether the predicted structure is correct or not. This server also gives the Ramachandran plot for the specified structures predicted.

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WETLAB TECHNIQUES

The extraction apparatus used to retrive the proteins from the considered leaf tissue of the Gossypium(Cotton) plant consists of the following equipment:

❖ Glass tubes and rods to extract the tissue from the leaves of the cotton plant

The leaf tissues should be taken in an order according to the number of the samples of their planting in the glass tubes and should be smashed nicely to get its tissue proteins out from the samples. To do this extraction procedurewe need to prepare certain solutions [3]. They are to be studied as follows:

- **1X Sample Buffer:** Take 50ml., of 10X sample extraction buffer,dilute it to 500ml., by adding D/W. Use 500ul or 1ml., per sample. Store at 4^{o}c.

- **10X buffer A(for 1Lit):**Mix Buffer A(premix) in 800ml of Milli_Q/Distilled water. Add 1ml., of solution – A per 1000ml., using Milli-Q/Distilled water.

- **1X Buffer A:** Take 100ml., of 10X buffer A,dilute it to 1L by adding D/W.

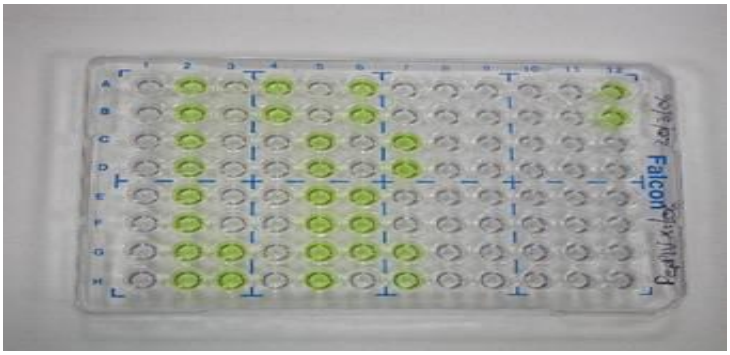
Sample preparation

For seed extract: Imbibe cotton seeds overnight in water. Remove seed cotton and place the seed in a microcentrifuge tube and add 500ul or 1ml., 1X sample extraction buffer. Crush with a pistle for 30sec., Use 50ul of this extract of sampling.

For leaf extracts: Punch out 3-4 leaf discs with a mcf tube by placing a leaf between the lid and the tube opening and closing the lid onto the leaf. Add 500ul or 1ml., 1X sample extraction buffer. Crush with a pestle for 30sec., Use 50ul., of this extract for sampling.

ELISA Apparatus:

After extracting the proteins from the leaf tissue, we need to keep them in the ELISA plate and follow certain steps by adding some buffer solutions.



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1 , 94	-	B	-	BLANK
2 , 95	-	P	-	POSITIVE
3 , 96	-	N	-	NEGATIVE

After keeping the samples in the ELISA plate, we need to keep the plate in the ELISA Reader for the positive and negative results of the protein presence in the samples considered.

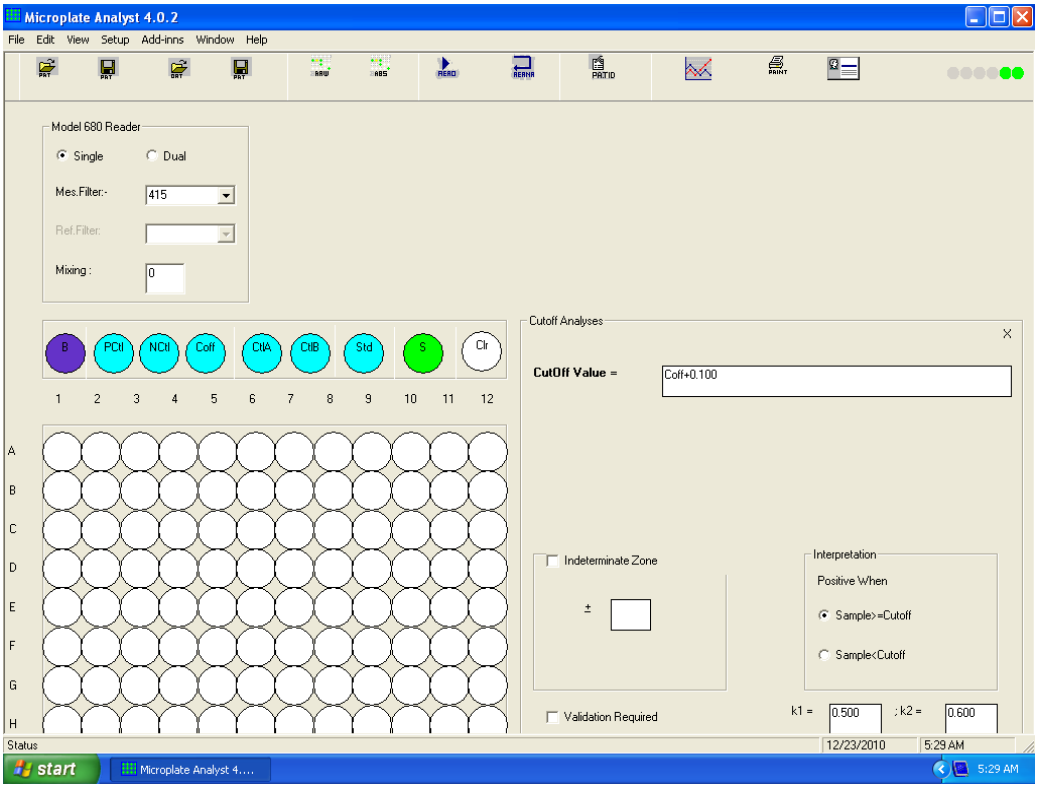
ELISA READER

From the considered samples, we need to keep them in the ELISA Reader and give a certain absorbance value to get the results.

Cutoff value =((1st blank reading + 2nd blank reading)/2) + 0.1

Among the obtained results, we need to check the values of the readings. Then, we have to note the values . If the values are more than the cutoff value, then we can conclude that the sample contains the specified protein and if it is less than that value, the protein is not present in the considered sample.

ELISA SOFTWARE



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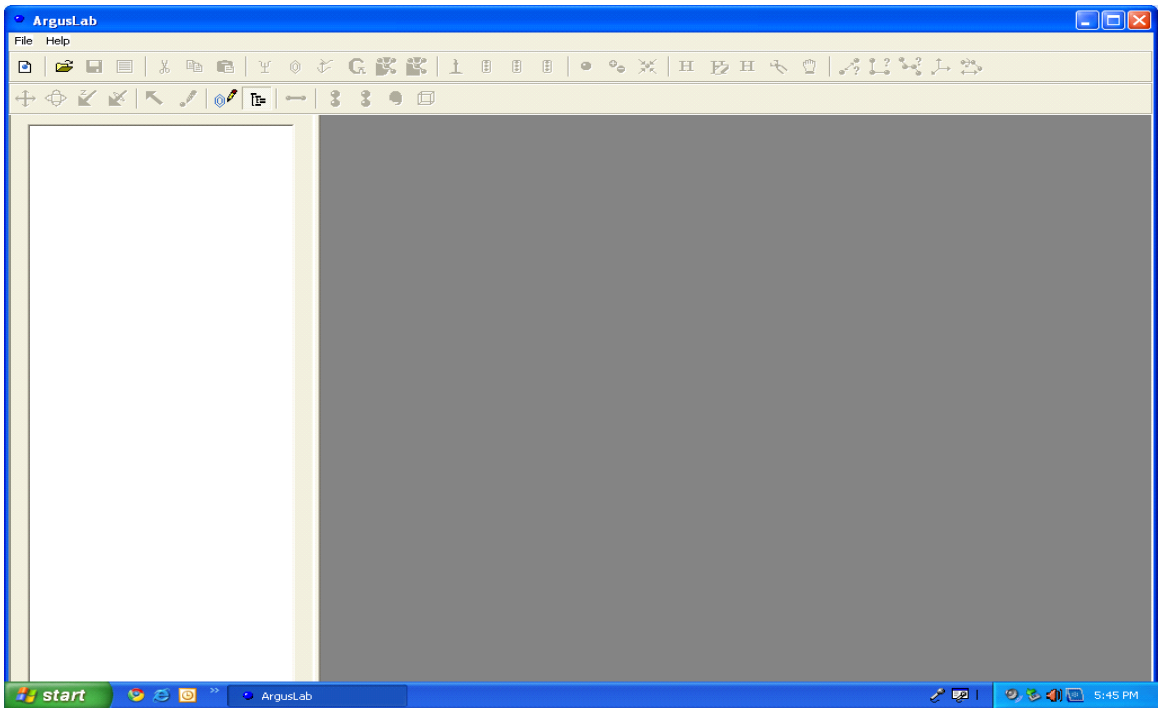
This is a microplate analyst. After analyzing all the samples in the ELISA plate and then keep it in the ELISA Reader, then, we can get the readings required for the considered samples.

TOOLS USED

ARGUS LAB

ARGUSLAB is a freely available tool in the online. It is used to do the valency satisfaction of the protein molecules, their minimization. It is mainly used to dock the target and ligand molecules. So that, it gives the poses of different conformations of the molecules. In that, we have to select the first best pose, which is having the minimum energy. The snapshot of the ARSUSLAB is as follows:



GRAMM Server:

This is the Web interface to our current protein docking software made available to the public. This software is different from the original GRAMM, except that both packages use FFT for the global search of the best rigid body conformations.

This server will ignore any small ligands or other non-protein molecules in the input files. It is designed exclusively for docking pairs of protein molecules.

You can submit input files and parameters to this web server and the docking simulation will be run on our computer cluster.

When the results are ready, they will be saved in a temporary directory on the web server and the link to that directory will be sent to user.

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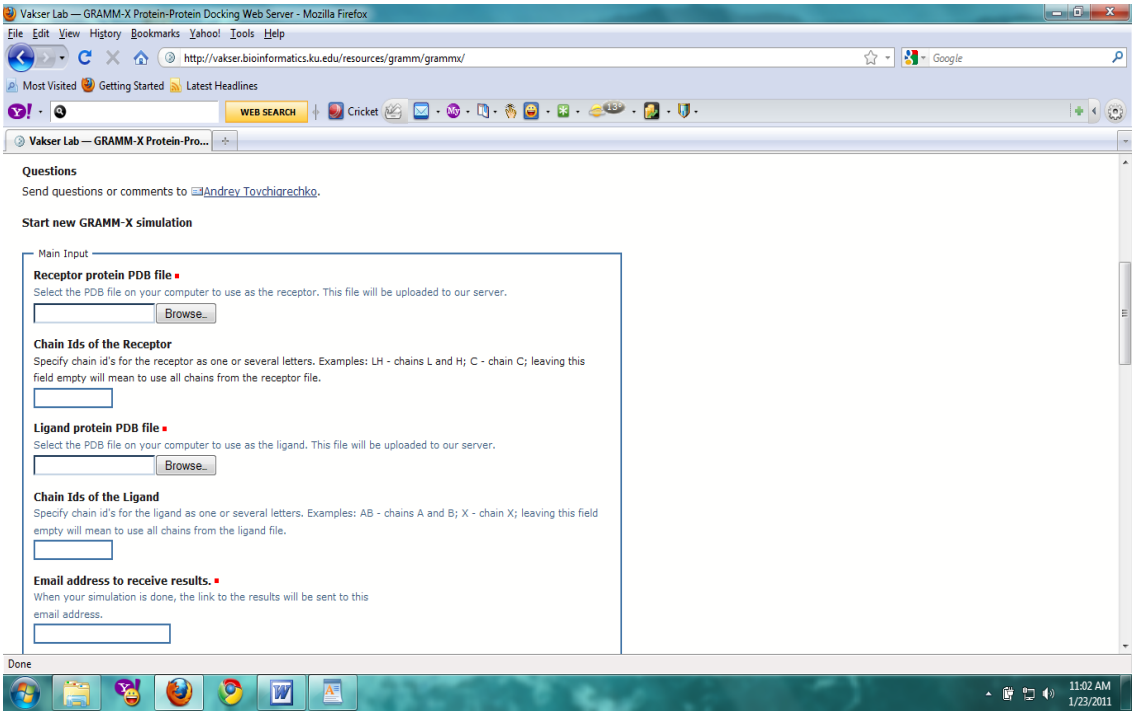
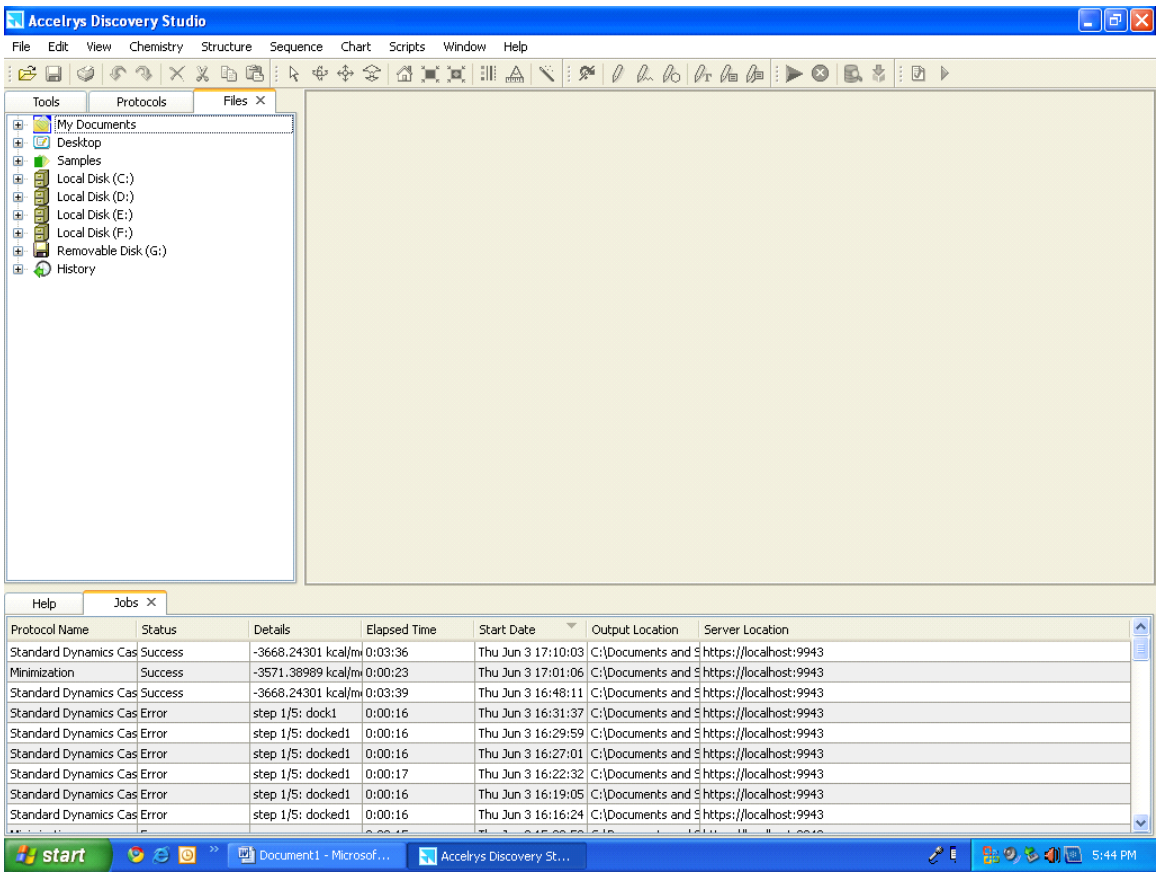


ACCELRYS DISCOVERY STUDIO

It is a commercial software. It is used to calculate the various energies of the doked molecules. First, we have to apply the force field which is suitable to the loaded molecule and then, we have to perform the simulation studies like, minimization, standard dynamics. With the obtained results, we can findout the best suited drug to the selected target protein. The snapshot of the ACCELRYS STUDIO is as follows:



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ALGORITHMS

The algorithms used in the simulation study work are **STEEPEST DESCENT, CONJUGATEGRADIENT, LEAPFROG ALGORITHM**. They can be explained in the following way:

STEEPEST DESCENT

The method of **steepest descent** or **stationary phase method** or **saddle-point method** is an extension of Laplace's method for approximating an integral, where one deforms a contour integral in the complex plane to pass near a stationary point (saddle point), in roughly the direction of steepest descent or stationary phase. The integral to be estimated is often of the form:

$$F(\lambda) = \int_{\gamma} f(z) e^{\lambda S(z)} dz,$$

where $\lambda > 0, \lambda \to +\infty$ is a large parameter, γ is a contour in the complex z-plane, and the functions $f(z)$ and $S(z)$ are holomorphic in a domain D containing γ. The zeros of $S'(z)$ are called the saddle points of $S(z)$. The essence of the method is as follows. The contour γ is deformed to a contour $\tilde{\gamma}$ with the same end-points and lying in D and such that $\max_{z \in \tilde{\gamma}} \operatorname{Re} S(z)$ is attained only at the saddle points or at the ends of $\tilde{\gamma}$.

CONJUGATE GRADIENT

The **conjugate gradient method** is an algorithm for the numerical expression of particular systems. The conjugate gradient method is an iterative method, so it can be applied to sparse systems that are too large to be handled by direct methods such as the Cholesky decomposition. Such systems often arise when numerically solving patial differential equations.The conjugate gradient method can also be used to solve unconstrained optimization problems such as energy minimization.

The biconjugate gradient method provides a generalization to non-symmetric matrices. Various nonlinear conjugate gradient methods seek minima of nonlinear equations.

Suppose we want to solve the following system of linear equations.

$$\mathbf{Ax} = \mathbf{b}$$

where the *n*-by-*n* matrix **A** is symmetric(i.e., $\mathbf{A}^T = \mathbf{A}$), positive definition (i.e., $\mathbf{x}^T\mathbf{Ax} > 0$ for all non-zero vectors **x** in $\mathbf{R}^n$)

LEAPFROG ALGORITHM

Leapfrog integration is a simple method for integrating differential equations, particularly in the case of a dynamical system. The method is known by different names in different disciplines. In particular, it is similar to the **Velocity Verlet** method, which is a variant of Verlet integration. Leapfrog integration is equivalent to calculating positions and velocities at interleaved time points, interleaved in such a way that they 'leapfrog' over each other. For example, the position is known at integer time steps and the velocity is known at integer plus half time steps.

Leapfrog integration is a second order method and hence usually works better than Euler integration, which is only first order. Unlike Euler integration, it is stable for oscillatory motion, as long as $dt < 1 / \omega$.

$$r(t + \delta t) = r(t) + v\left(t + \frac{1}{2}\delta t\right)\delta t$$

$$v\left(t + \frac{1}{2}\delta t\right) = v\left(t - \frac{1}{2}\delta t\right) + a(t)\delta t$$

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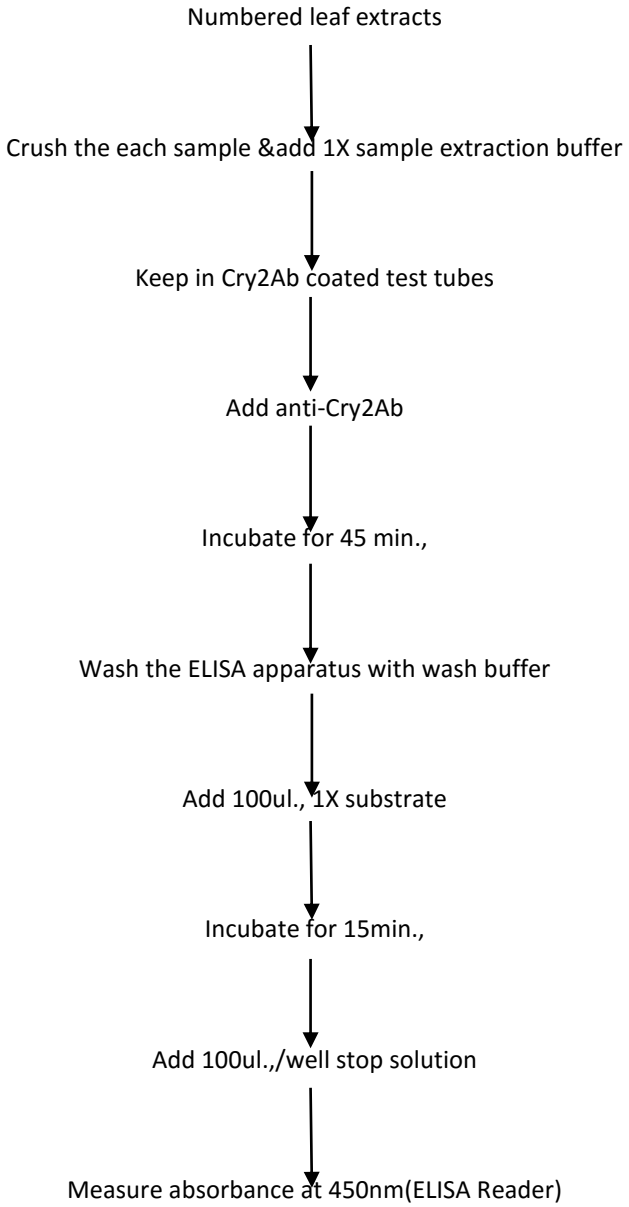
In this algorithm, the velocities are first calculated at time *t+1/2dt*; these are used to calculate the positions, *r*, at time*t+dt*. In this way, the velocities *leap* over the positions, then the positions *leap* over the velocities. The advantage of this algorithm is that the velocities are explicitly calculated, however, the disadvantage is that they are not calculated at the same time as the positions. The velocities at time *t* can be approximated by the relationship:

$$v(t) = \frac{1}{2}\left[v\left(t - \frac{1}{2}\delta t\right) + v\left(t + \frac{1}{2}\delta t\right)\right]$$

METHODOLOGY

Leaf samples from 90 Bt cotton plants have been taken for the checking of presence of Cry1Ac and Cry2Ab proteins which are endotoxins. To do this, we need to use extraction apparatus and ELISA Reader.Here, we need to perform series of steps to check the presence of required Cry1Ac & Cry2Ab proteins.

SERIES OF STEPS TO EXTRACT PLANT PROTEIN



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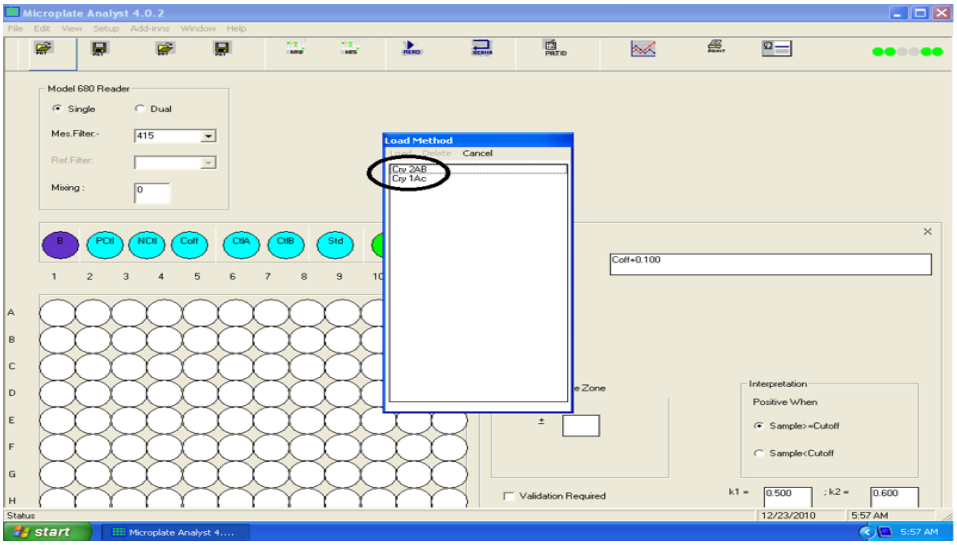
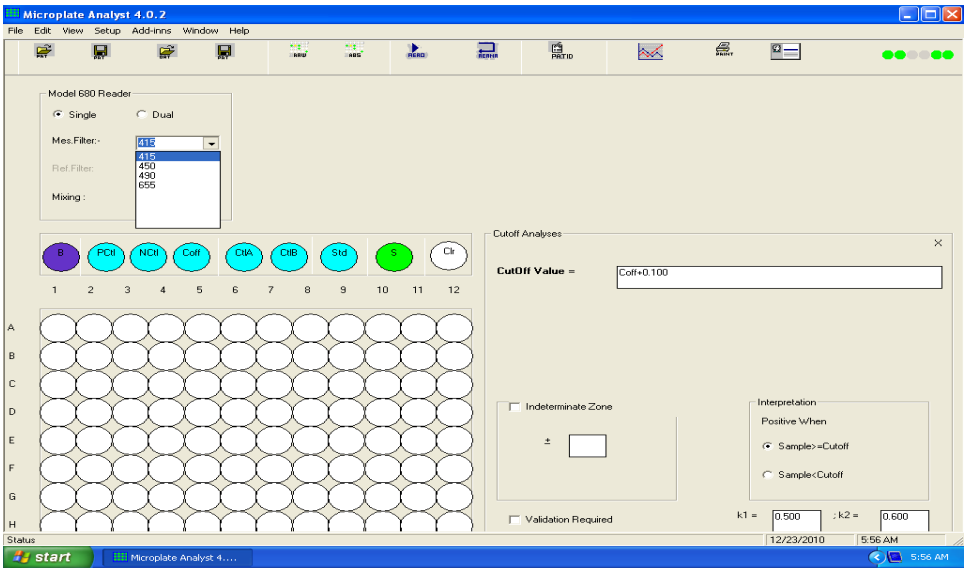
Criteria for a valid ELISA test / Classification of positives

After doing all the steps in the protocol, we need to keep the microplate in the ELISA Reader, then we need to keep it give specific options for each protein in the microplate analyst software.

The below is the snapshot to select the specific protein to be tested which gives us the result in the considered samples.



Sample wells with all reagents added except seed/leaf extract(blank wells), should have an absorbance value below 0.150 at 450nm at the time of taking the reading.



The below is the snapshot of results of ELISA Reader of Cry1Ac protein.

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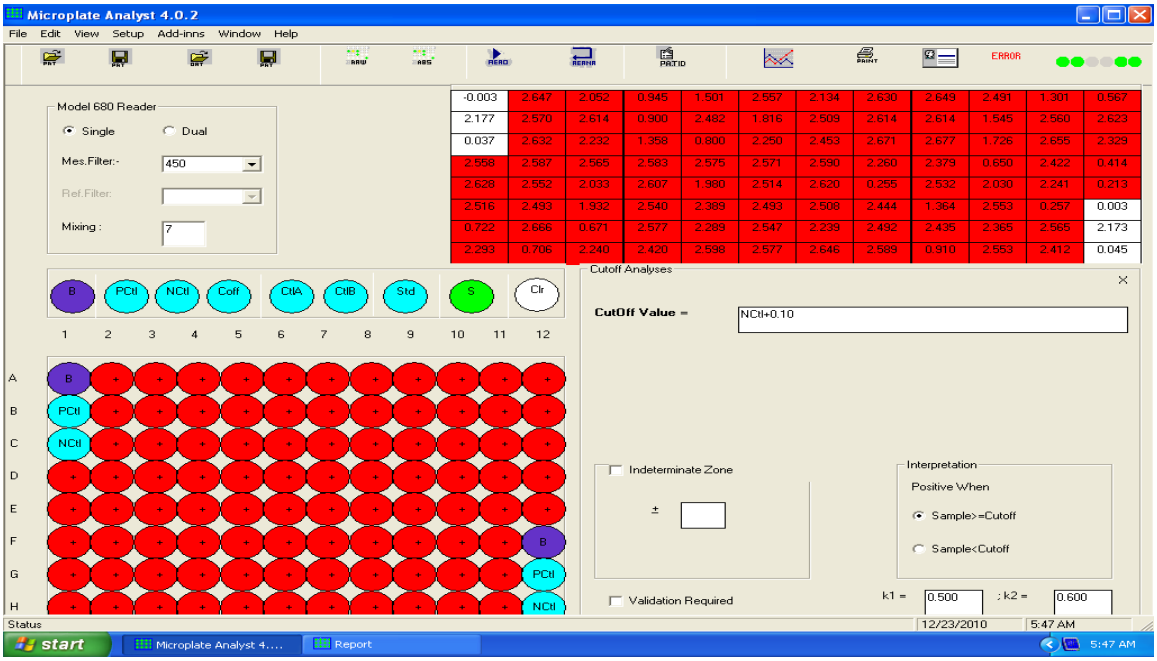
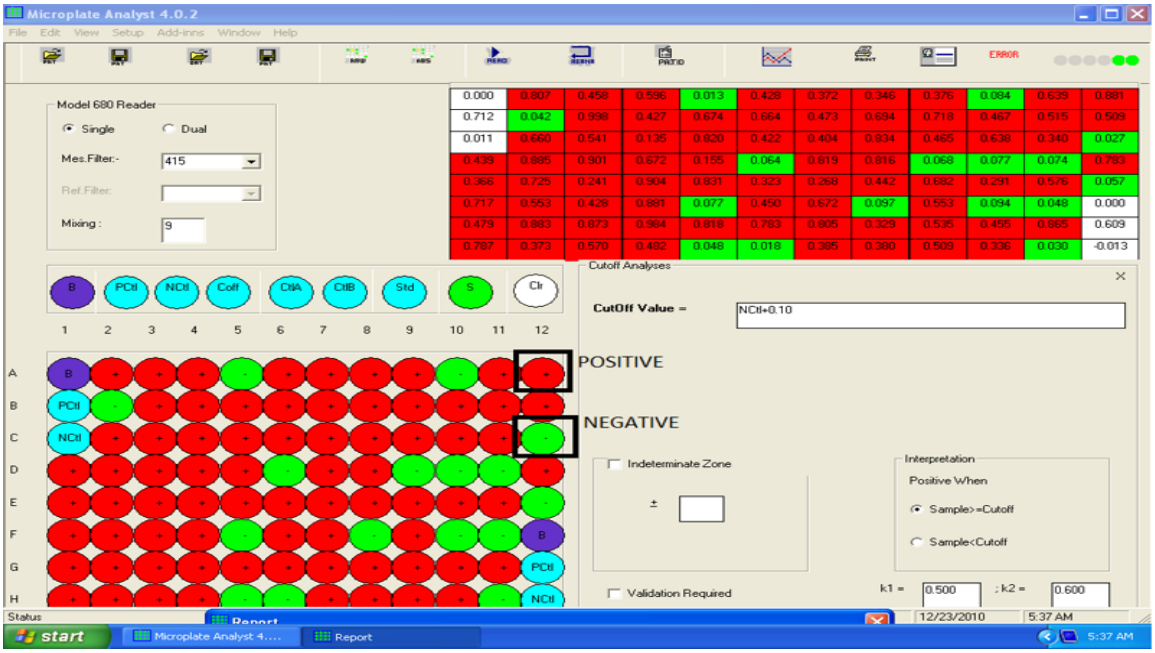


The below is the snapshot of the results of ELISA Reader of Cry2Ab protein.



The above are the results of respected proteins, Cry1Ac & Cry2Ab

The **Violet**coloured blocks - **Blank**
The **Red**coloured blocks - **Positive** results
The **Green**coloured blocks - **Negative** results
The **Blue**coloured blocks - **Positive & Negative**blocks(test tube)

Later, we have to consider only the positive samples and send them in individual groups to the experimental lanes for the testing of their susceptance and resistance. According to that we can decide, the presence of which protein gives more resistance to the Cotton crop.

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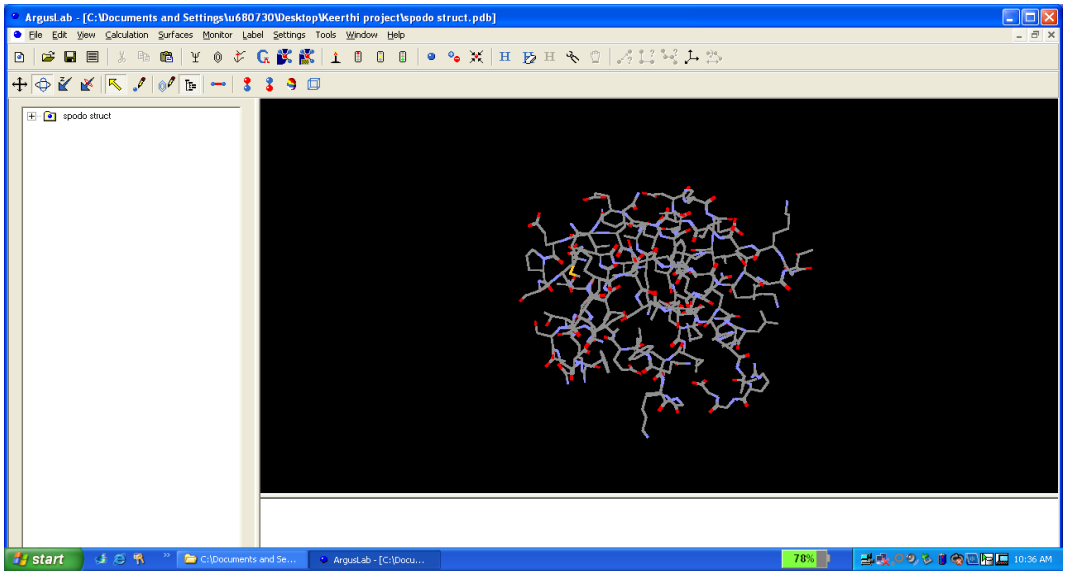
We can also prove these results by testing the proteins after docking them with the disease causing protein i.e., **6-aminobutarate aminotransferase of Spodoptera**. Atlast, we can decide which protein gives the best result of giving resistance to the Cotton crop [5].

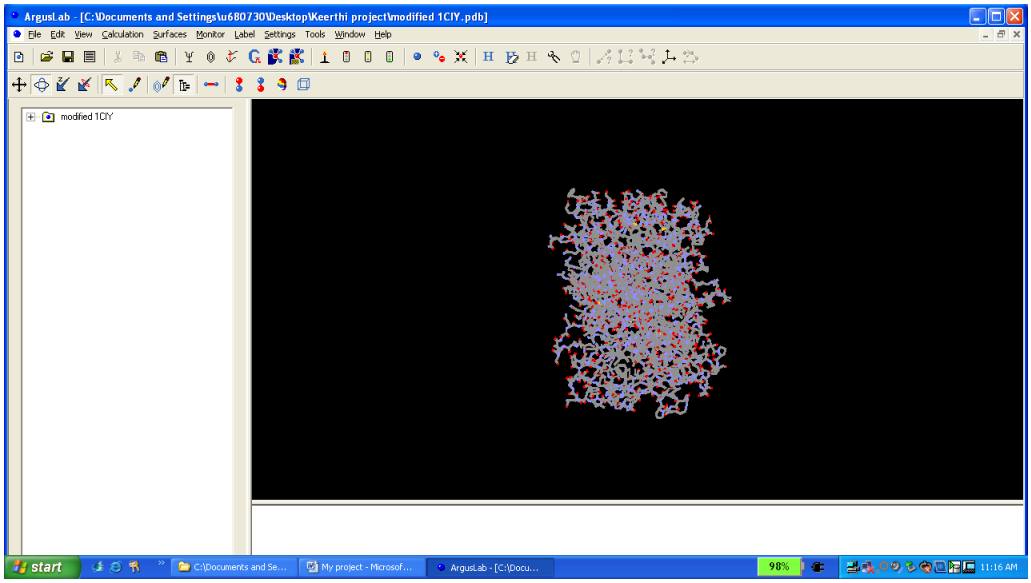
DRYLAB EXPERIMENTAL METHODOLOGY:

For the experimental proof of the resistance based problem of Bt Cotton genes/proteins, we have to take the diseases causing protein i.e., 6aminobutarate aminotransferase from the Spodoptera larva and dock them with the Cry1Ac and Cry2Ab proteins.

First, we need to retrieve the pdb structures of the disease causing protein i.e., 6aminobutarate aminotransferase of Spodoptera larva pests of the cotton plants and then we have to remove the hydrogen atoms from those structures and load them in the ARGUS LAB to view the structure of the protein.The retreived and loaded structure of the Spodoptera larva which causes more infection to the Gossypium species is as follows:



Then, we have to do the homology modelling for the Cry1Ac and Cry2Ab proteins. We can do this my retreiving the sequence from the **Uniprot** and if we give that particular sequence in the **SWISS MODEL**, the results of the structures are sent to the mail ID. Then, we can load in the ARGUS LAB and can view them after removing hydrogen atoms from them.



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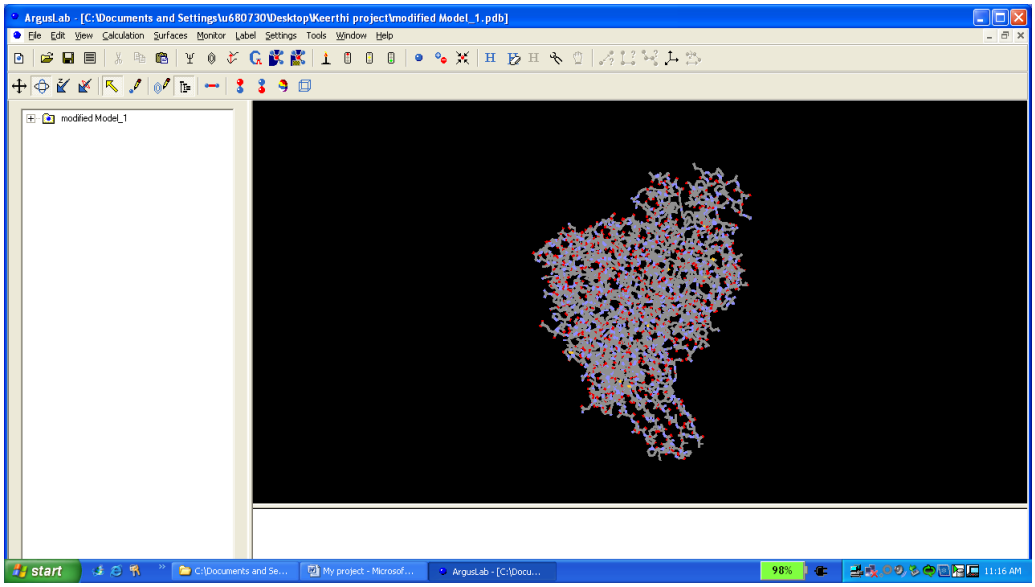

Later, we have to dock the Cry1Ac & Cry2Ab individually with the disease causing proteins of Lepidoptera and Spodoptera using an online server GRAMMx. If we, we browse and give the pdb files to the server, we can get the docked structures to the mail ID. Then, we can load these docked structures in the **Accelrys Discovery STUDIO** to view the structures and calculate the minimised energies.

RESULTS & DISCUSSION

The SAVS results for the **6-aminobutarate aminotransferase** after Homology modelling is as follows:

The above result of **Ramachandran plot** says us that the most of the aminoacids are in the most probable region. So, we can say that this structure is accepted.

The results in the wetlab and expriment to check the presence of the **Cry1Ac** protein in the concerned plant samples is as follows:

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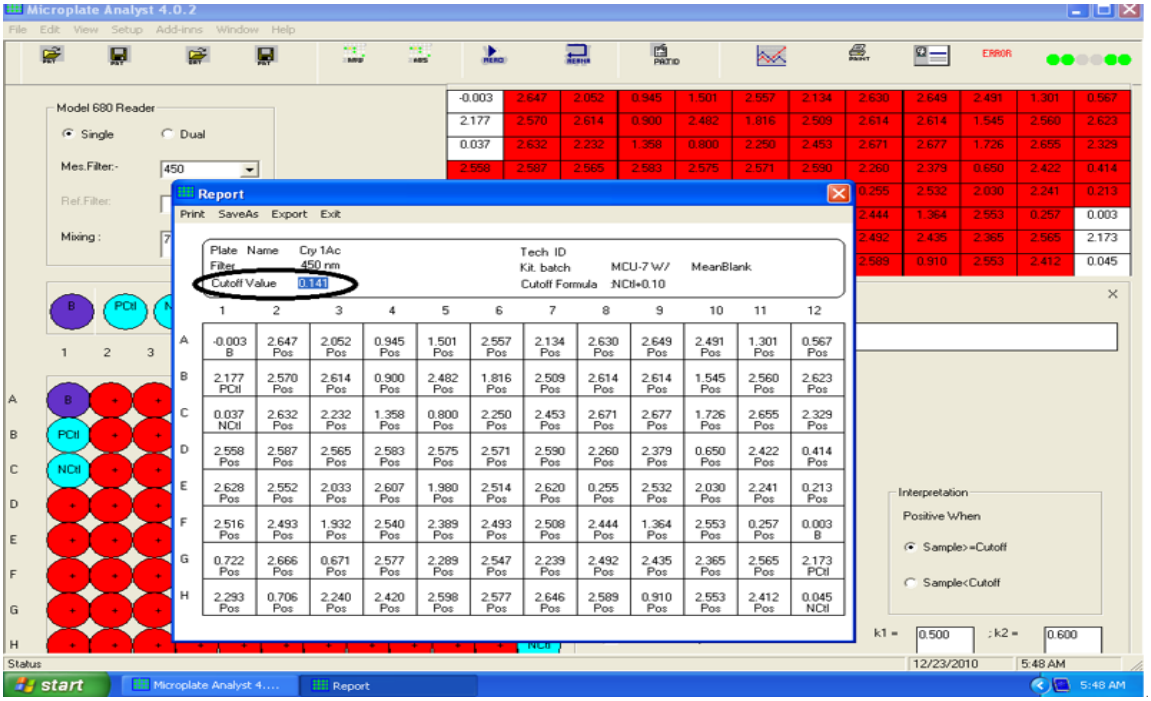

Here, the cutoff value is **0.141** and we can note that the values above it are positives and below it are negatives in respect to the Cry1Ac protein.

The results of the positives of Cry1Ac in the considered samples and which shows resistance towards the Spodoptera larva are as follows:

BLANK	**NO 6-POS**	**NO 14-POS**	**NO 22-POS**	**YES 30-POS**	**NO 38-POS**	**NO 46-POS**	**NO 54-POS**	**NO 62-POS**	**YES 70-POS**	**NO 78-POS**	**NO 86-POS**
POSITIVE	**YES 7-POS**	**NO 15-POS**	**NO 23-POS**	**NO 31-POS**	**NO 39-POS**	**YES 47-POS**	**YES 55-POS**	**NO 63-POS**	**NO 71-POS**	**NO 79-POS**	**NO 87-POS**
NEGATIVE	**NO 8-POS**	**NO 16-POS**	**NO 24-POS**	**NO 32-POS**	**NO 40-POS**	**YES 48-POS**	**YES 56-POS**	**NO 64-POS**	**NO 72-POS**	**NO 80-POS**	**YES 88-POS**
No 1-POS	**YES 9-POS**	**NO 17-POS**	**NO 25-POS**	**NO 33-POS**	**YES 41-POS**	**NO 49-POS**	**NO 57-POS**	**NO 65-POS**	**YES 73-POS**	**NO 81-POS**	**NO 89-POS**
No 2-POS	**YES 10-POS**	**NO 18-POS**	**NO 26-POS**	**YES 34-POS**	**NO 42-POS**	**NO 50-POS**	**NO 58-POS**	**YES 66-POS**	**NO 74-POS**	**NO 82-POS**	**NO 90-POS**
Yes 3-POS	**NO 11-POS**	**NO 19-POS**	**YES 27-POS**	**NO 35-POS**	**NO 43-POS**	**NO 51-POS**	**YES 59-POS**	**YES 67-POS**	**NO 75-POS**	**NO 83-POS**	**BLANK**
N0 4-POS	**NO 12-POS**	**NO 20-POS**	**NO 28-POS**	**YES 36-POS**	**NO 44-POS**	**NO 52-POS**	**NO 60-POS**	**NO 68-POS**	**YES 76-POS**	**YES 84-POS**	**POSITIVE**
NO 5-POS	**YES 13-POS**	**YES 21-POS**	**NO 29-POS**	**NO 37-POS**	**YES 45-POS**	**YES 53-POS**	**NO 61-POS**	**NO 69-POS**	**NO 77-POS**	**NO 85-POS**	**NEGATIVE**

The above table shows us the results about the resistance of **Cry1Ac** protein in the considered samples. The other results in the blocks are the numbers of the samples and the **"POS"** gives us the conformation about the presence of **Cry1Ac** protein.

Here, we can conclude that Cry1Ac protein is not the exact Crystal protein for giving the resistance to the Gossypium species because we can see that even some samples contain Cry1Ac protein, they didn't show the resistance over Spodoptera larva.

The results in the wetlab and expriment to check the presence of the **Cry2Ab** protein in the concerned plant samples is as follows:

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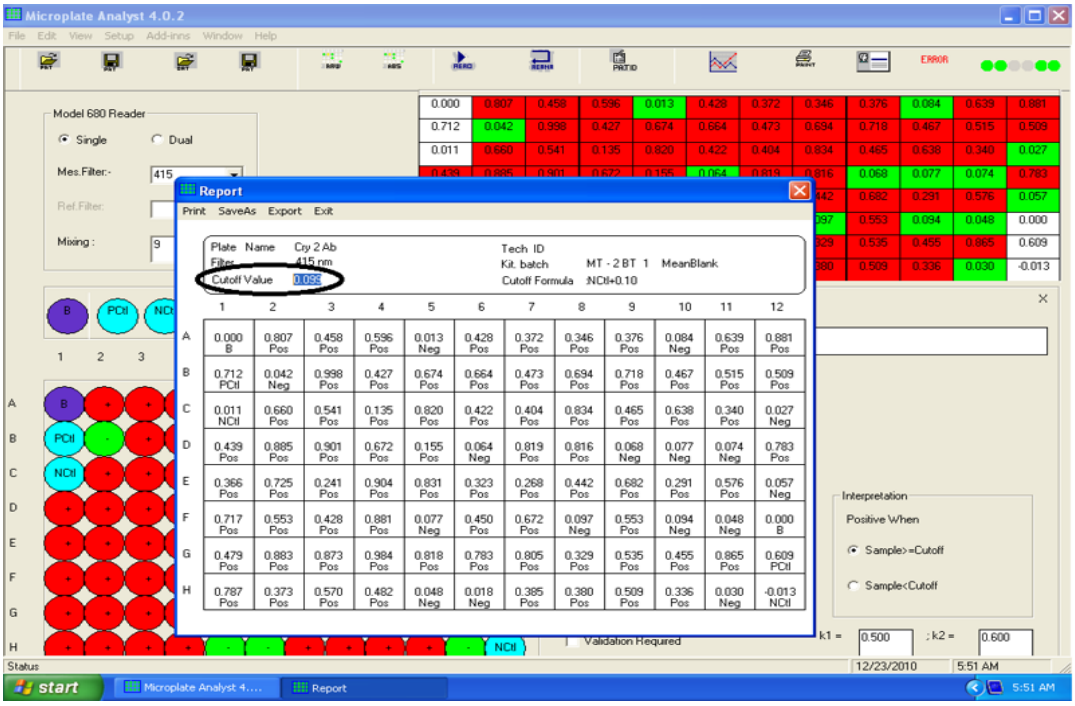

Here, the cutoff value is **0.099** and we can note that the values above it are positives and below it are negatives in respect to the **Cry2Ab** protein.

The results of the positives of **Cry2Ab** in the considered samples and which shows resistance towards the Spodoptera larva are as follows:

BLANK	**YES 6-POS**	**YES 14-POS**	**YES 22-POS**	**30-NEG**	**YES 38-POS**	**YES 46-POS**	**YES 54-POS**	**YES 62-NEG**	**YES 70-POS**	**YES 78-POS**	**YES 86-POS**
POSITIVE	**7-NEG**	**YES 15-POS**	**YES 23-POS**	**YES 31-POS**	**YES 39-POS**	**YES 47-POS**	**YES 55-POS**	**YES 63-POS**	**YES 71-POS**	**YES 79-POS**	**YES 87-POS**
NEGATIVE	**YES 8-POS**	**YES 16-POS**	**YES 24-POS**	**YES 32-POS**	**YES 40-POS**	**YES 48-POS**	**YES 56-POS**	**YES 64-POS**	**YES 72-POS**	**YES 80-POS**	**88-NEG**
YES 1-POS	**Yes 9-POS**	**YES 17-POS**	**YES 25-POS**	**YES 33-POS**	**41-NEG**	**YES 49-POS**	**YES 57-POS**	**65-NEG**	**73-NEG**	**81-NEG**	**YES 89-POS**
YES 2-POS	**Yes 10-POS**	**YES 18-POS**	**YES 26-POS**	**Yes 34-POS**	**YES 42-POS**	**YES 50-POS**	**YES 58-POS**	**YES 66-POS**	**YES 74-POS**	**YES 82-POS**	**90-NEG**
YES 3-POS	**YES 11-POS**	**YES 19-POS**	**YES 27-POS**	**35-NEG**	**YES 43-POS**	**YES 51-POS**	**59-NEG**	**Yes 67-POS**	**75-NEG**	**83-NEG**	**BLANK**
YES 4-POS	**YES 12-POS**	**YES 20-POS**	**YES 28-POS**	**YES 36-POS**	**YES 44-POS**	**YES 52-POS**	**YES 60-POS**	**YES 68-POS**	**YES 76-POS**	**YES 84-POS**	**POSITIVE**
YES 5-POS	**YES 13-POS**	**YES 21-POS**	**YES 29-POS**	**37-NEG**	**YES 45-NEG**	**YES 53-POS**	**YES 61-POS**	**YES 69-POS**	**YES 77-POS**	**85-NEG**	**NEGATIVE**

The above table shows us the results about the resistance of Cry2Ab protein in the considered samples. The other results in the blocks are the numbers of the samples and the "POS" gives us the conformation about the presence of Cry2Ab protein and "NEG" says us about the absence of the Cry2Ab protein

Here, we can conclude that Cry2Ab protein is not the exact Crystal protein for giving the resistance to the Gossypium species because we can see that even some samples contain Cry2Ab protein, they didn't show the resistance over Spodoptera larva.

The final result of the drylab experiments can be seen in Accelrys STUDIO with the minimised energies of the docked structures.

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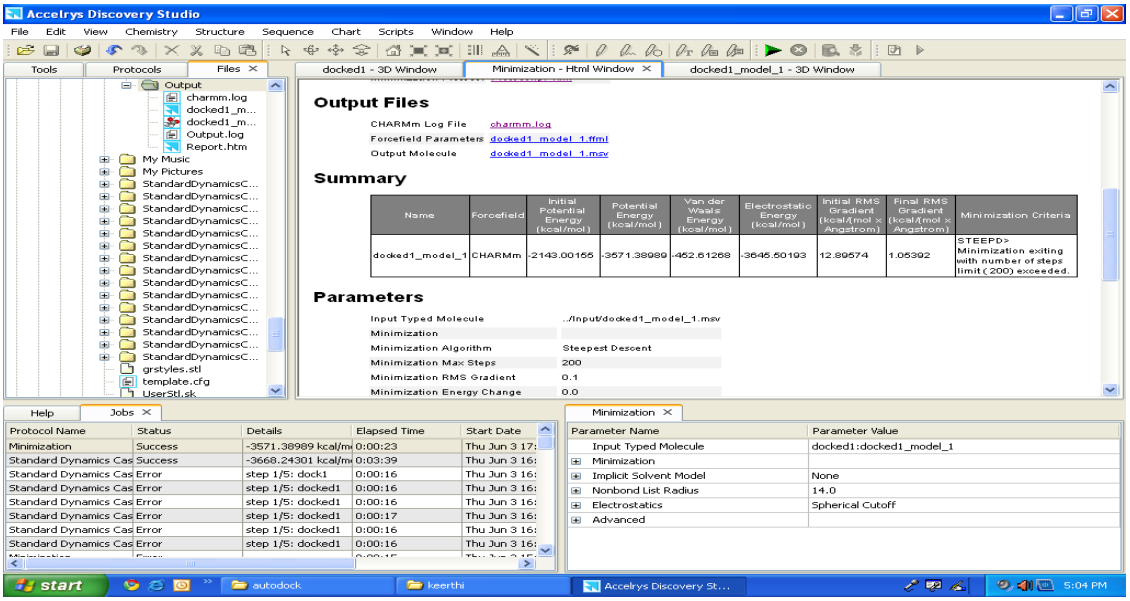
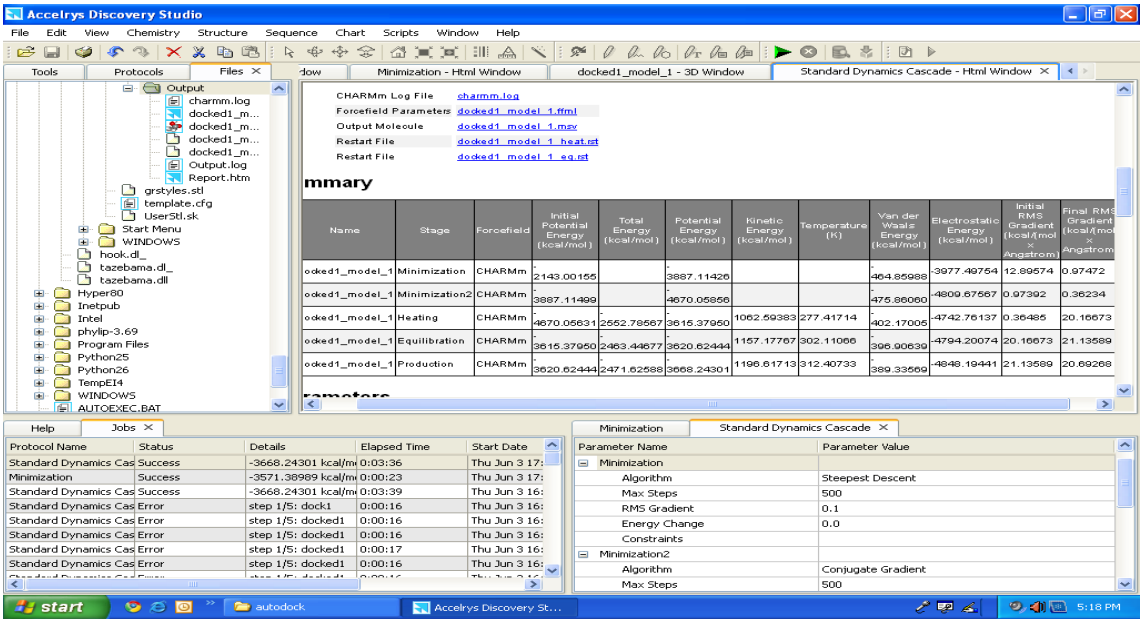
The below are the results in concerned with the calculated energies of the minimised docked structres. The best energies i.e., the lowest energies are obtained to the docked structures of the Lepidopteran and Spodopteran larvae with the Cry2Ab protein. The below snapshots shows the results for potential, kinetic, vanderwaals, electrostatic energies using the forcefield **CHARMm** and the minimisation algorithm, **Steepest Descent and ConjugateGradient and LEAPFROG ALGORITHM**. The best results can be shown as follows:





CONCLUSION

Since, cotton is a very important commercial plant in the present day world, we need to increase its resistance over pests and insects. Among these the larva of Spodoptera are more infectious. So, we can control these by transferring resistance giving genes, Cry1Ac and Cry2Ab to the cotton genome and prove this task.

We proved this both in practical and experimental ways by wetlab and drylab techniques. By these experiments, we can conclude that the Cry1Ac and Cry2Ab proteins in the Bt Cotton plant can give more and more resistance to the crop both from the Spodopteran larva. The wetlab and research work in the fields and the drylab works by docking these structures with the 6-aminobutarate aminotransferase gave us the same result about the Crystal proteins, i.e., Cry2Ab is the best in giving resistance to the Cotton crop among the known Crystal proteins.

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