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# Case study: Agri-biotech

## Design of Biosensor for herbicide

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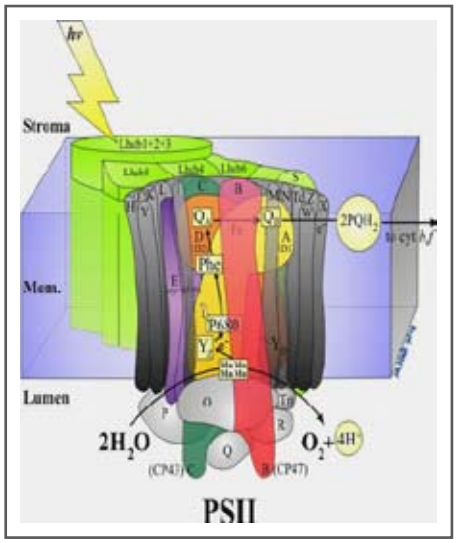
Customer type

**A leading research organization**

Software modules

**BioPredicta**

**VLife Engine**




Photosystem II

Application

**Biosensor design**

Techniques

**Protein structure analysis**

**Protein - ligand docking**

**Binding energy analysis**

### Background:

The widespread use of herbicides in agriculture, industry and urban areas poses a threat to the entire ecosystem. Due to its effectiveness and low cost, atrazine (6-chloro-N2-ethyl-N4-isopropyl-1,3,5-triazine-2,4-diamine) is one of the most widely used herbicides throughout the world but also a persistent contaminant in aquatic ecosystems. Therefore there is significant interest in the development of biosensors that can provide reliable and rapid in-situ measurement of herbicide concentration in the environment.

Atrazine inhibits photosynthesis by interrupting the photosynthetic electron transport chain at the photosystem II (PSII) site. PSII is a multi-subunit pigment-protein complex embedded in the thylakoid membrane of oxygen-evolving phototrophs. The reaction center of PSII consists of two protein subunits D1 and D2. Plastoquinone QB and QA are substrates of the subunits D1 and D2 respectively. Atrazine competitively inhibits QB site in D1 subunit, thereby imparting its herbicide effect. The D1 and D2 sites are in contact with each other and hence inhibitor present in QB site of D1 influences QA binding in D2.

The customer requirement was to provide insights for design of biosensor for atrazine using modeling techniques in order to avoid significant experimental effort.

### Design considerations:

For achieving above goal, we have performed *in-silico* mutation studies for all the residues within active site of D1 and D2 subunits (defined by residues within 5.5 $A^0$ from QB and QA binding site). The model for D1 and D2 subunits were obtained by using homology modeling tool of BioPredicta. Each residue was mutated by remaining 19 residues and conformational space of mutated residues was explored.

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VLifeMDS

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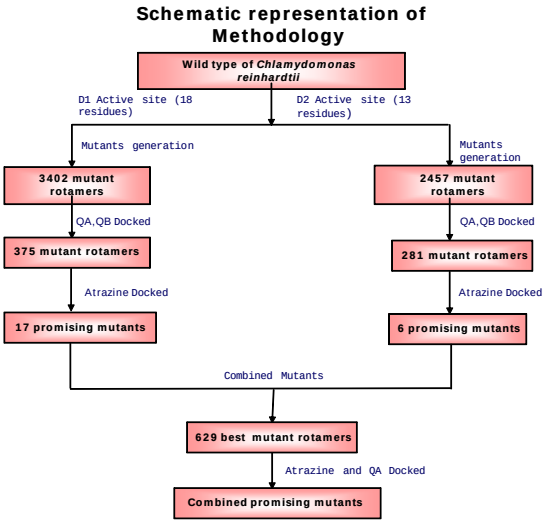
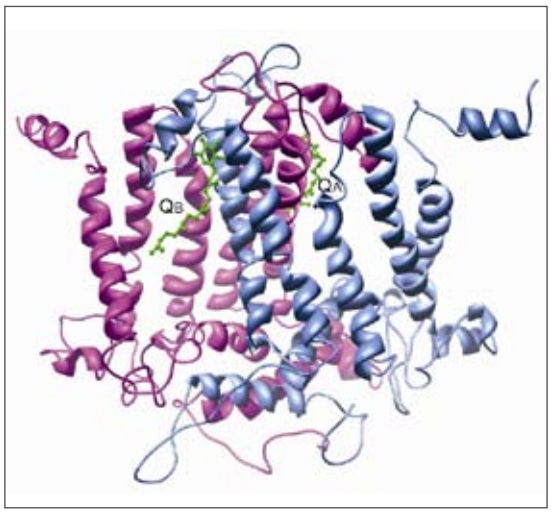



Workflow of project




Model of *C. reinhardtii* D1 and D2 proteins.

## Project work:

The three-dimensional structure of *C. reinhardtii* D1 and D2 proteins was obtained using homology modeling using the crystal structures of *T. elongatus* as templates (PDB code: 2AXT, Loll et al., 2005). *T. elongatus* D1 and D2 proteins display 87% and 89% amino acid sequence identity with *C. reinhardtii* D1 and D2 proteins, respectively.

- Each residue mutation generates 189 rotamers of the remaining 19 residues. A total of 18 single site mutant residues produced 3402 rotamers in the D1 active site.
- 13 single site mutant residues produced 2457 rotamer structures in the D2 active site.

Simultaneous mutations at D1 and D2 sites were also explored. Atrazine was docked in D1 site and plastoquinone in D2 active site. The binding energies of mutated and wild type proteins were compared to find out promising mutations.

## Validation studies:

To validate the computational approach undertaken in the present study, docking simulations were performed on mutants of the D1-D2 proteins for which experimental data concerning resistance to the herbicides atrazine or metribuzin, i.e. A251I, F255Y and L275F was available.

Docking studies of atrazine and plastoquinone were performed on 189 different rotamer structures of the *C. reinhardtii* D1 A251I mutant. Results obtained with the five best rotamer structures indicate that binding energy for atrazine increases several kcal/ mol in the mutant protein (lower affinity) in agreement with the experimental evidence that mutation of Ala251 to Ile in the D1 protein confers herbicide resistance and photoautotrophic growth.

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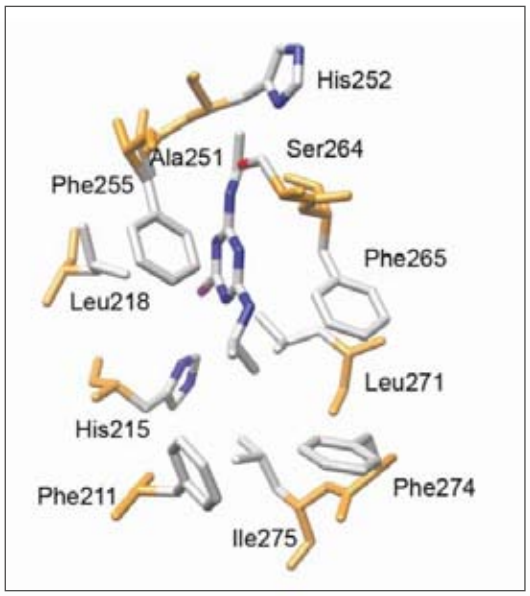
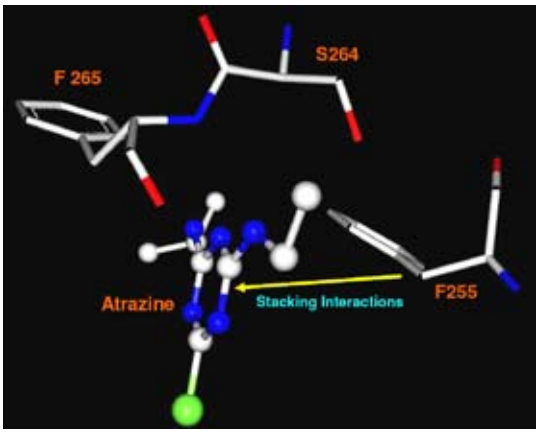



Atrazine binding site in wild type D1




Pi-Stacking interactions of Atrazine with F255 (mutant L267V)

Results obtained by docking studies of atrazine on F255Y and L275F mutant structures indicated that the first mutation leads to a strong increase in the binding energy value for atrazine (lower affinity) while the latter leads to a decreased binding energy value (higher affinity). This is in line with the experimental evidence that mutation of Phe255 to Tyr confers resistance to atrazine, but not to metribuzin, while mutation of Leu275 to Phe confers resistance to metribuzin, but not to atrazine. These validation studies support approach taken for the mutation analysis.

## Result analysis:

Comparison of atrazine binding energy in wild type D1 with the corresponding mutants of D1 active site residues led to suggestion of promising D1 mutations

Comparison of QA binding energy in wild type D2 with corresponding mutants of D2 active site residues led to suggestions for promising D2 mutations.

Comparison of binding energies of atrazine and QA for simultaneous mutations of D1 and D2 site with corresponding wild type is sufficient to suggest possible mutations that may enhance atrazine binding in D1 while retaining QA in D2.

Modeling studies provided necessary clues for design of biosensor for the herbicide. Results obtained lead to the identification of a limited set of mutations that can significantly increase atrazine binding affinity and thus represent an important step towards the exploitation of D1-D2 proteins for the development of highly specific herbicide biosensors.

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