# Playing Catchup : Adapting Computer Aided Drug Design Techniques to Agriculture

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GENOME SCIENCE AND TECHNOLOGY
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SCALE-IT
Scalable Computing and Leading Edge Innovative Technologies

Introduction:

The plant pathogen *Phytophthora capsici* threatens close to 1 billion dollars worth of crop annually. It has a broad host range covering the *Solanacea , Cucurbitacea,* and *Leguminosea.* There are currently only two fungicides available for control and the pathogen develops resistance to these chemicals after a few generations of exposure. The pathogen is a late blight meaning that it manifests late in the growing season creating harvest loss and the loss of all inputs required for cropping.(Fertilizer , fuel, time, etc.)

This has caused much of the *Phytophthora* community to begin looking at genetic causes of infection in order to develop cropping plants with genetic resistance. As a result of these studies a class of proteins termed RxLR effectors have been described and shown to be vital in the pathogens ability to successfully infect hosts. These proteins have also been demonstrated to associate with specific lipids in cell membranes facilitating entry into the host cell. A reasonable conclusion was drawn that the RXLR portion of these effectors was responsible for binding. This hypothesis was tested and reported as true[1]. Shortly after this first report a second lab demonstrated that a positively charged patch in a structured portion of the protein was in fact responsible. [3]

We have used molecular docking software to validate the second groups report and to begin dissecting the molecular mechanisms responsible for the binding of the protein to the lipid head. By understanding these mechanisms we will be able to help develop targeted pesticides that will block the effectors ability to reach the host cell cytoplasm.

Methods and Materials:

Publicly available structures of the RxLR effectors Avr3a and Avr3a4 were downloaded from the protein data bank.(http://www.rcsb.org/) The sequences of binding knockout mutants reported in Yaeno *et al* [3] were then modeled to these structures using the homology modeling server Phyre2 (http://www.sbg.bio.ic.ac.uk/phyre2). The structure of the lipid ,Phosphatidylinositol 3-phosphate(PI3P), was obtained from the lipidomics gateway (http://www.lipidmaps.org/) and edited to remove the hydrophobic tails.

The software suite autodock tools (autodock.scripps.edu) was used to define the search parameters for docking simulations and all dockings were performed using the autodock vina program[2].

The first set of simulations was to determine whether PI3P associated with the binding site described in Yaeno *et al* or at the RxLR motif. This was done by simulating docking where the entire protein was considered and comparing these results to the results of limiting docking to just the RxLR motif and the positive patch (Figure 1). These simulations indicate that the binding pocket described by Yaeno *et al* is energetically the most favorable region for binding.

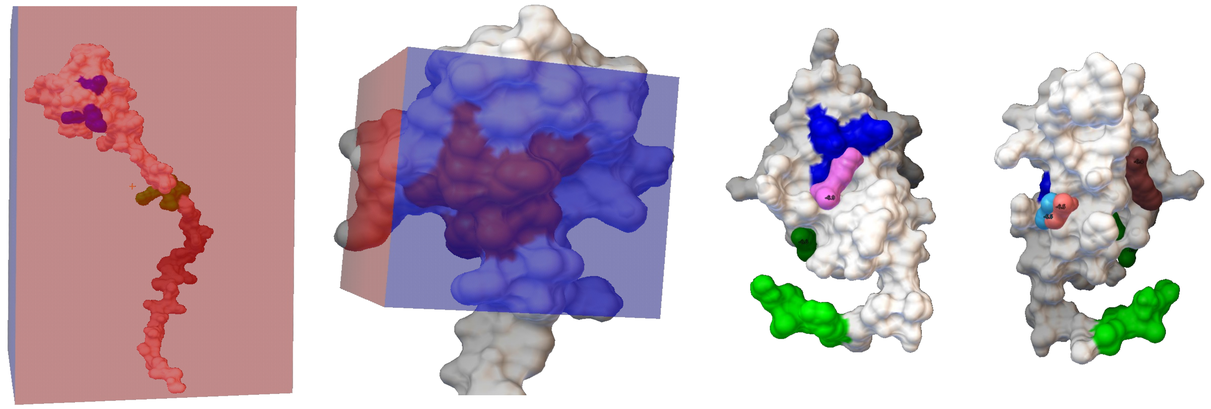


**Figure 1**. Design and results of “blind” docking. A) The global docking search space RXLR and Positive patch are highlighted in green and blue respectively. B) Positive patch reported as being vital for binding in Yaeno et al and the search space used for verification. C) Best energy results of docking from the global docking search. The best energy docking resides at the positive patch reported by Yaeno et al. (Pink).

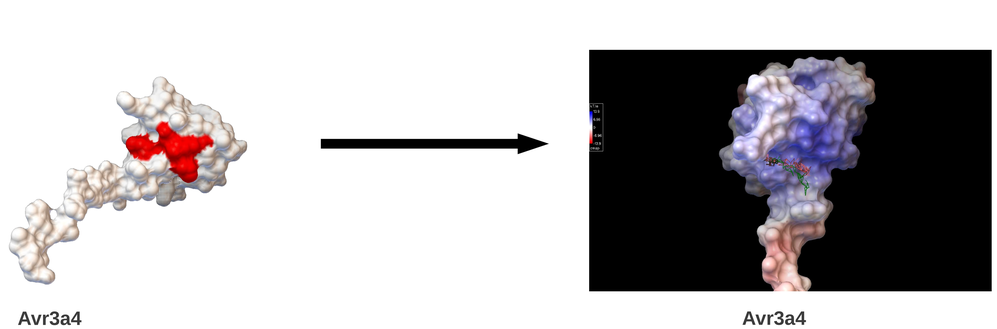



**Figure 2.** Avr3aKI & Avr3a4. The lysine residues thought to be crucial for binding PI3P are highlighted in red. The mutant proteins have the glutamate mutation highlighted in orange.

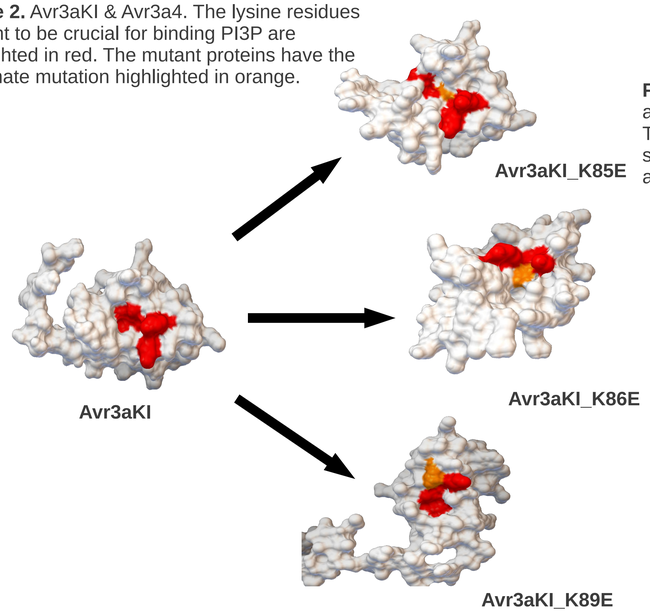
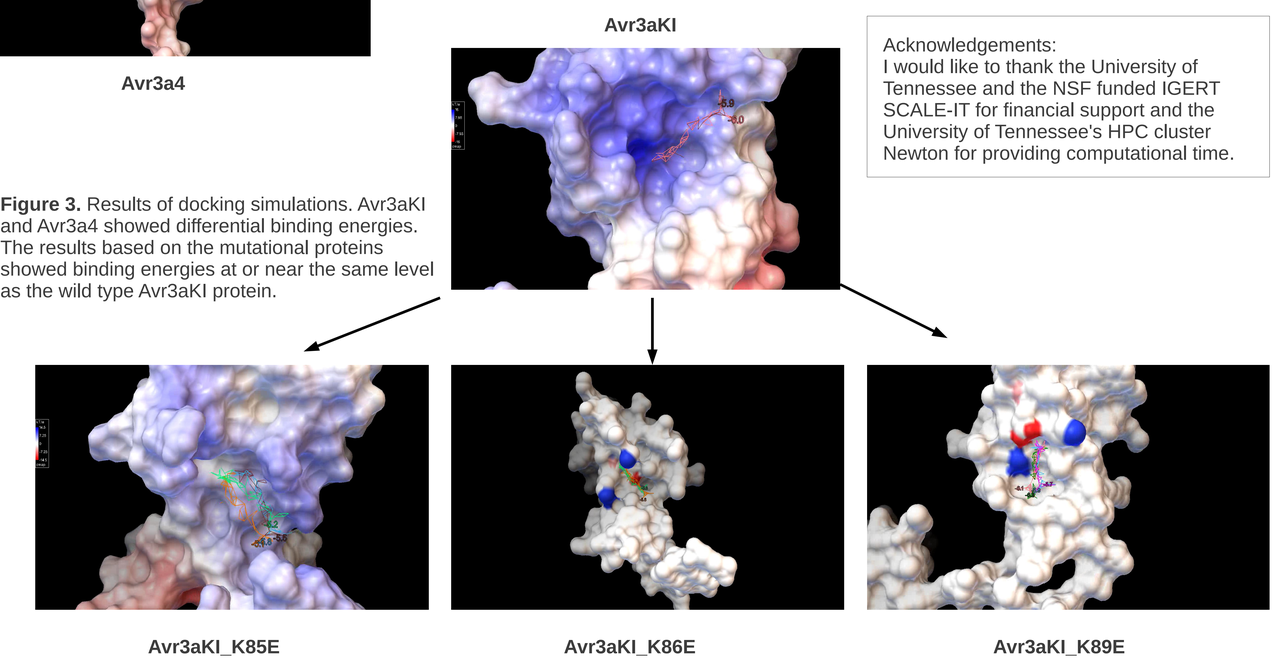



**Figure 3.** Results of docking simulations. Avr3aKI and Avr3a4 showed differential binding energies. The results based on the mutational proteins showed binding energies at or near the same level as the wild type Avr3aKI protein.




Methods and Materials (Continued):

The mutational studies reported in Yaeno et al were simulated in order to provide lower bounds of energy of binding in pockets that are known not to bind. A natural mutant of Avr3aKI , Avr3a4, that does not show an affinity to PI3P was also included in this set of simulations. (Figure 2 and Figure 3)

Discussion :

It is our hope that these simulations will help clear the waters on what portions of the protein are responsible for binding lipids of the host cell membrane. These simulations and models will also prove invaluable in providing data on new chemicals that may prove useful in combating this pathogen in the field.

While initial simulations were promising, mutational simulation results were problematic. Results could be due to the over simplification of the system. (A single lipid floating in a vacuum is not a lipid membrane.) In an effort to remedy this situation molecular dynamic simulations are currently under way.

Future Directions:

Even though the simulations of mutations proved problematic I am of the mindset that beginning to screen compounds that will bind tightly to these pockets will still be useful. I am also creating a bank of simulations that will cover multiple genetic variants in the protein effectors. ( I have currently found ~350 genetic variants in a single sequenced isolate of the pathogen.) I am hopeful that these simulations will become a valuable tool for the community and agriculture as a whole.

Acknowledgements:
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Citations:

1) Kale SD, et al. (2010) External lipid PI3P mediates entry of eukaryotic pathogen effectors into plant and animal host cells. Cell 142:284–295

2) Trott, O. and Olson, A. J. (2010), AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. J. Comput. Chem., 31: 455–461.

3) Yaeno T, et al. (2011) Phosphatidylinositol monophosphate-binding interface in the oomycete RXLR effector AVR3a is required for its stability in host cells to modulate plant immunity. Proc Natl Acad Sci USA 108:14682–14687