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# How 'the crowd' is tackling a silent killer

Citizen scientists around the globe are uniting to make **AFLATOXIN** a thing of the past.

## AUTHORS

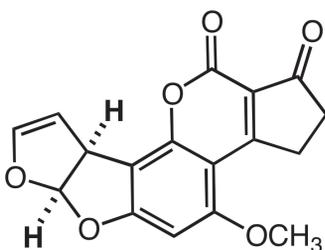
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## AFLATOXIN, A SILENT KILLER IMPACTING PEOPLE AND CROPS AROUND THE WORLD

It is difficult to believe, but 4.5 billion people globally are chronically exposed to a harmful group 1 carcinogen through their food<sup>1</sup>, yet they're either unaware or without alternative options. This little known, wide reaching poison is called aflatoxin. The health impacts of aflatoxin are staggering: it is the most potent naturally occurring liver carcinogen we know, and is estimated to play a part in up to 28% of liver cancer cases globally<sup>2</sup>. Furthermore, consuming aflatoxin-contaminated food is associated with stunting in children, damage to the immune system, maternal anaemia and mortality.

Several approaches for managing and degrading aflatoxins are currently in practice, but none are widely considered



Chemical structure of aflatoxin B<sub>1</sub>.



*Aspergillus flavus* - the fungus that produces aflatoxin - growing on corn.

to be effective. Scientists think an enzyme can be created to attack and degrade aflatoxin, which would decrease toxicity by several orders of magnitude. As a result, a group of uncommon collaborators including Mars, Incorporated, the University of California, Davis, Thermo Fisher Scientific, the University of Washington, Northeastern University, the Partnership for Aflatoxin Control in Africa (PACA) and the United Nations Food and Agriculture Organization (UN FAO) have come together to tap into the power of online, gamified protein folding with hopes that it can expedite progress towards a solution.

Aflatoxins are a type of mycotoxin - poisonous natural products made by certain fungi that can evoke a toxic response when consumed, even in low concentrations. These mycotoxins are largely invisible compounds that can grow in or on almost all grains and groundnuts. It's

estimated that mycotoxins contaminate at least one quarter of food crops around the world, such as maize, tree nuts, cassava, millet, peanuts, several spices and even animal feeds<sup>3</sup>. Under certain circumstances, the toxin can contaminate crops in the field and in storage, with fungi growth most pronounced post-harvest, especially in moist, warm conditions.

In mature economies, expensive monitoring and advanced food safety technologies track aflatoxin levels to keep it out of the food chain. Where the toxin level is above legal limits, the food goods are disposed of. Although total food loss and waste figures are not known, it is widely understood that aflatoxin infestation causes a significant volume of food waste globally.

Aflatoxin disproportionately affects people in poorer countries. Even though food aflatoxin limits are set, they often go unenforced

partly due to under-regulated food manufacturing and reduced access to monitoring technology. As a result, the impacts of aflatoxin are felt strongly in poorer countries.

## THE POWER OF PROTEIN FOLDING

Proteins are the molecular building blocks for almost every process in living things and are responsible for structure, function and regulation in our bodies. Whilst deoxyribonucleic acid (DNA) is the posterchild of biology and provides the instructions for life in the form of our genetic code, proteins are essential in mobilizing this genetic information so that organisms can develop, reproduce and live. In the past few decades, biotechnology has empowered scientists to remodel fundamental molecules through a process called 'protein folding' in an effort to solve a range of medical challenges, such as diabetes, arthritis and cancer.

Proteins are made up of strings of amino acids that determine a protein's unique three-dimensional (3D) structure, and can be rearranged into different shapes. In fact, there are so many possibilities for potential proteins that it makes the quantity of stars in the universe look tiny. The unique structure of each protein dictates its function, and so scientists can aim to change a protein's behaviour by rearranging its structure.

Scientists and medical doctors recognize the tremendous opportunity that protein folding could offer to treat many medical conditions. Whilst most scientific discoveries in this realm typically take place in private labs, some scientists have identified the



An example of the image that a gamer will see when folding proteins on the Foldit platform.

opportunity to leverage the power of 'the crowd' to explore new protein structures at an expedited rate. One such example is Foldit, an online science crowdsourcing platform that can be played by anyone with a computer and an imagination. It challenges participants to play with 3D protein puzzles that scientists can use to tackle real world medical challenges. Launched in 2008 by David Baker and scientists from the University of Washington, Foldit has already resulted in multiple crowdsourced breakthroughs. For example, in only three weeks gamers solved the structure of an enzyme involved in the reproduction of human immunodeficiency virus (HIV) – a mystery that had stumped scientists for more than a decade<sup>4</sup>. In the past five years, scientists at the University of Washington's Baker Lab have used protein structures fashioned by Foldit participants around the world that act as biosynthetic catalysts<sup>5</sup>, fight coeliac disease<sup>6</sup> and treat anthrax infections<sup>7</sup>.

The process of digital protein folding can be modelled using computer algorithms and software, thanks to advancements in the field of computational biology pioneered by Michael Levitt, Martin Karplus and Arieh Warshel, winning them the Nobel Prize in Chemistry in 2013.

But why can't computers solve the protein folding challenges on their own? Classical computers cannot sample all 3D orientations

at once, because this requires enormous amounts of processing power. Classic protein modelling software is programmed to try combinations essentially at random – named the 'random-walk' approach. There are of course supercomputers, some of which are specifically built for protein modelling – take IBM's Blue Gene or D.E. Shaw's Anton as examples – yet these are still unable to sample sufficient structural space to be effective in searching the designable protein landscape. With essentially infinite possible protein orientations and thousands of medical challenges, using random-walk algorithms on supercomputers doesn't quite cut it. Crowdsourcing offers a pathway to investigate new structural possibilities beyond what is traditionally explored.

#### HUMAN INTUITION AT AN UNPRECEDENTED SCALE

Since the Foldit Aflatoxin Challenge launched on United Nations' World Food Day 2017, gamers from across the globe have been competing to design enzymes that can tackle aflatoxin. In less than one year, players have designed more than 1.6 million models to potentially degrade aflatoxin. The time these players have taken to morph the 3D molecules equates to approximately 80,000 player hours – that's equivalent to the labour force of over 100 full-time employees working on the problem for one year.

The gamers are diverse – ranging from 12 to 80 years old. What connects them is the innate spatial reasoning abilities of the human brain, enabling gamers to tackle some of the hardest problems in biology today. Humans are geared to detect patterns in everything they experience, and Foldit takes advantage of this puzzle-solving intuition. In fact, within the community of aflatoxin gamers, some of the highest scoring Foldit accounts are owned

by individuals with no scientific training. This approach provides a scale and diversity of protein-folding capabilities that cannot be achieved by an individual lab.

#### HOW THE FOLDIT AFLATOXIN COLLABORATION WAS ESTABLISHED

Mars believes that food safety is fundamental to food security. Food security is defined by the UN FAO as all people at all times having access to sufficient, safe and nutritious food. Because Mars is a global business, it recognized the problem of aflatoxin and its impact on food supply around the world. In India, the company was rejecting up to 70% of the peanuts at the factory gate because of elevated levels of aflatoxin. There was no guarantee, however, that the rejected crops were not entering the food supply chain elsewhere. To address this little known but devastating food safety issue, a group of uncommon collaborators came together to tackle this challenge.

recognized the opportunity to use the Foldit gaming platform to leverage the scientific capabilities of the partnering scientists and the skills and imaginations of gamers globally.

If a solution to taking down aflatoxin is produced from this collaboration, it will be freely available to anyone in the world. Mars and partners have committed that all player designs will be available in the public domain, free from patents, to maximize the positive impact that this project could have on global food safety.

#### SYNTHETIC BIOLOGY AS A FORCE FOR FOOD SAFETY

Over the past year there have been 12 gaming rounds of the aflatoxin puzzle released on the Foldit platform. After each game round, the best scoring models are picked for the next round of the process – analysis. Scientists at the Siegel Lab at the University of California, Davis analyze the



A young scientist playing the Foldit Aflatoxin Challenge at the 68th Lindau Nobel Laureate Meeting.

On UN World Food Day 2017, a unique partnership was born between University of California, Davis, Thermo Fisher Scientific, the University of Washington, Northeastern University, the Partnership for Aflatoxin Control in Africa (PACA), the United Nations Food and Agriculture Organization (UN FAO) and Mars, Incorporated. The group

protein structures for their amino acid sequence and send the information to Thermo Fisher Scientific. Thanks to advances in synthetic biology technologies, the amino acid information is translated and optimized into biology's digital code – DNA, the software of life. DNA is physically produced by Thermo Fisher Scientific's synthetic



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*There isn't time to wait to solve the problems that aflatoxin creates, because it's already impacting the health and well-being of 4.5 billion people. At Mars we want to improve food safety and security for people around the world, including the most in need and low-income populations.*

biology team, leveraging their proprietary oligo and gene synthesis capabilities that encode for the newly designed proteins. Future DNA synthesis runs will use Thermo Fisher's miniaturized semiconductor-based nucleic acid synthesis platform. This technology can generate 35,000 individually selectable oligos manufactured at the same time, which are then stitched together to make up the code for the enzyme.

Once created, the synthesized DNA molecules are sent back to the Siegel Lab to see if – when expressed and folded into real proteins – they have the ability to detoxify aflatoxin. The scientists are particularly interested in targeting aflatoxin's susceptible lactone ring, because this allows the synthesized DNA to form an enzyme capable of performing lactone hydrolysis on aflatoxin B<sub>1</sub> under industrial conditions where only water needs to be present to perform the desired chemistry<sup>8</sup>. Chemical degradation of this lactone ring through enzymatic hydrolysis has the potential to decrease aflatoxin mutagenicity by more than 400-fold<sup>9</sup>.

### THE ROAD AHEAD

Although there is still much work to be done, scientists are already seeing forward momentum. With more promising amino acid strings produced with every iteration, it

is only a matter of time before synthetic biology produces an enzyme that can do the job.

The Foldit platform not only provides the protein designs, but facilitates knowledge exchange between citizen scientists and the researchers behind the scenes. What is promising is the way in which gaming rounds learn; they fine tune the digital protein to enable gamers to reach more meaningful 3D orientations. There exists a symbiosis between the Foldit gamers and the Siegel Lab, because each new round builds off feedback from the last to optimize the puzzle and the solution selection parameters. Recent rounds have allowed gamers greater movement of the aflatoxin molecule and trimmed the scaffold protein area, enabling Foldit players to design an active site that creates a more tightly bound molecule.



Researchers in the Siegel Lab at UC Davis, Wai Shun Mak (pictured above in background) and Ryan Caster (project lead, pictured above in foreground), analyzing proteins for aflatoxin activity.

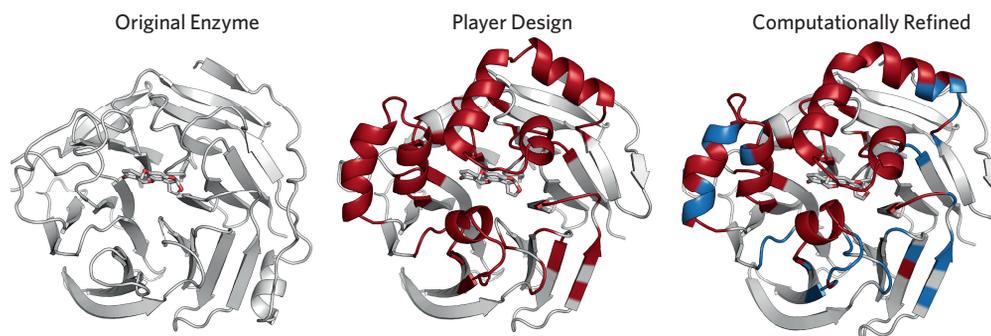
As the project continues to evolve, more and more game players are taking part in the movement. This is not only an effort to neutralize aflatoxin, it is also a step towards inclusive citizen science and open dialogue between a diverse set of collaborators.

What we are seeing is a new approach to one of society's grand challenges. The partners are tackling one of the most persistent carcinogens in a way that has not been attempted since aflatoxin was discovered in the 1960s. If efforts are successful, the positive impacts will largely be felt in young economies, where aflatoxin unfairly burdens society. Promisingly, the Foldit Aflatoxin Challenge has brought together a unique set of collaborators – industry, academia and the

general public – to advance scientific discovery and, ultimately, tackle the persistent threat of aflatoxin around the world.

### REFERENCES

- Williams, J. H. *et al. Am. J. Clin. Nutr.* **80**, 1106–1122 (2004).
- Liu, Y. & Wu, F. *Environ. Health Perspect.* **118**, 818–824 (2010).
- Marin, S., Ramos, A. J., Cano-Sancho, G. & Sanchis, V. *Food Chem. Toxicol.* **60**, 218–237 (2013).
- Khatib, F. *et al. Nature Structural & Molecular Biology* **18**, 1175–1177 (2011).
- Eiben, C. B. *et al. Nature Biotechnology* **30**, 190–192 (2012).
- Gordon, S. R. *et al. J. Am. Chem. Soc.* **134**, 20513–20520 (2012).
- Wu, S. J. *et al. J. Biol. Chem.* **286**, 32586–32592 (2011).
- Nicolás-Vázquez, I., Méndez-Albores, A., Moreno-Martínez, E., Miranda, R. & Castro, M. *Arch. Environ. Contam. Toxicol.* **59**, 393–406 (2010).
- Ehrlich, K.C., Moore, G. G., Mellon, J. E. & Bhatnagar, D. *World Mycotoxin J.* **8**, 225–233 (2015).



The stages of player protein designs on the Foldit platform. (Left: original enzyme. Centre: enzyme with gamer design. Right: enzyme with player design and computational refinements).