

# The Case of the “Tainted” Taco Shells: Advanced Edition

by

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## Part I – Taco Night

“Hi Dad, I’m home! Can Chris join us for supper?” Mark asked as he walked into the kitchen. “Practice ran over and he’s got to go straight to the fields and help his dad, but there’s always time for taco night, right?”

Dad lifted his head up from the newspaper. “Only if he likes tacos without the shells. Hi, Chris. Got a date for the prom yet?”

“Nope. Still looking, Mr. Schumer.”

Dad continued, “I went to Kroger today, and all of the Taco Bell shells were gone. The cashier said there was some sort of recall. And you know your sister can’t have the flour ones because of her wheat allergy.”

“Well, we’ve got some hot dog buns, so let’s imp-ro-vice!” Mark said with a flourish. “What’s up with the recall? I thought that only happened with bad hamburger?”

Dad folded the paper over and pointed to a headline. “It’s all in the genes. This article says the shells accidentally contained some genetically modified corn called StarLink. StarLink is a feed corn, but it isn’t approved for human consumption.”

“So the cows can eat it, but we can’t? That doesn’t make sense,” said Mark.

Chris jumped in. “StarLink hasn’t been proven to be safe for humans to eat—you know, not toxic or allergenic. That’s important, especially to your sister.”

“What’s important to me?” asked Michelle as she walked in and sat at the table. “Hi, Chris.”

“Oh... Hey, Michelle.” Chris was blushing. “We were just talking about, um, taco shells.”

Mark rolled his eyes.

Dad turned to Chris. “Chris, your family farms corn, right?”

“Right.”

“Well, why would your dad want to grow genetically modified corn?”

“Well, do you remember the year Mark helped us detassle?” Chris asked.

Mark winced. “That was the worst job ever. My hands felt like they were at a paper-cut convention!”

“That was the easy part.” Chris said. “What if you had to walk the fields twice a year and pull all the weeds? That’s what my dad had to do when he was a kid. Now he uses Roundup spray and Roundup Ready seeds. He sprays the field once and all the weeds die. But the crop is okay because the seeds have been genetically modified so the plants won’t be affected. The only bad thing is that the seeds are a little more expensive and you have to buy the spray, but it is still a lot cheaper than weeding by hand.”

“And my hands thank you,” Mark said, waving his hands and bowing.

Chris ignored Mark and continued. “My dad uses another seed that is resistant to insects. When I was ten years old, we almost lost the entire crop to corn borers, so Dad was pretty happy when this seed came out.”

“So how does it work?” Michelle asked, smiling.

“Well,” said Chris, his face still a little pink. “All I know is from talking to the seed salesman. He said the corn produces a bacterial protein that is toxic to insects. So when a bug chews on the plant, it dies.”

Michelle frowned. “Bacteria? You mean they’re loading up my food with extra chemicals and toxic proteins just to make life easier for farmers?”

“Wait a minute. Farming isn’t easy, Michelle. My dad had to get a second job just to keep the farm that’s been in our family for three generations!”

“Well, what about the butterflies?” Michelle asked, setting her jaw. “They’re related to corn borers. Are they killing them, too?”

“I don’t know, Michelle.” Chris was getting defensive. “Besides, what does that matter? Butterflies don’t eat corn!”

“But I do!” Michelle sputtered. “And I’m not gonna eat any of this Franken-Food if I can help it!”

Mark stuck his arms out forward and wobbled around. “Igor, it’s alive! ALIVE!!”

Dad looked up again. “Mark, grow up. Michelle, calm down. This could be a good thing. There’s another article in here about a company that is genetically modifying rice to include vitamin A. They think it will greatly reduce childhood blindness in developing countries.”

“That’s great,” Mark said, “but with all this modification and insect resistance I wonder what happens if the weeds become resistant to the Roundup?”

“Good question,” said Dad. “Let’s continue this over dinner. Mark, sit over here. Chris, why don’t you sit next to Michelle.”

### *Questions*

1. How is genetically modified corn made and detected?
2. How do genetic modifications give plants insect and herbicide resistance?
3. Is genetically modified corn safe?
4. Can farmers make a living without it?
5. Will Chris ask Michelle to the prom?

## Part II – Project Design

To understand the science behind the use of genetically modified foods, Chris, Michelle, and Mark ask our class to help them. Since there is a lot of material to understand, we will divide into three “interest groups.” The three interest groups are:

- Entomologists
- Farmers
- Immunologists

Each student in the class will be assigned to an interest group. Each group will read several primary literature papers to understand how transgenic plants are made and the biochemical issues of interest to their constituents and then hand in written answers to the common questions and their group questions.

Each group will also prepare a 15-minute oral presentation to be given in lab. In the oral presentation, you should integrate your written answers into a broader discussion of when or whether genetically modified plants should be used. Your written work will be due at the time of your oral presentation. Following the presentation, there will be an opportunity for questions from the other groups.

Your grade will be 50% for the group written work, 40% for the group presentation, and 10% for your participation in the discussions of the other interest groups.

### Common Questions (all groups answer these in their written answers)

Read:

- Pöpping, Bert. “Are you ready for [a] Roundup?” *Journal of Chemical Education*, June 2001, 78: 752–756.

Then, answer the following questions:

1. Figure 1 in the article shows a pair of scissors cutting out a gene of interest. What kind of enzyme do the scissors represent? What techniques might you use to confirm that the gene of interest has been inserted into the plasmid (the circular piece of DNA)?
2. Besides the gene of interest, what other DNA sequences must be inserted into the plant to make it express a protein of interest?
3. Describe the three most common genetic modifications of plants and why each modification has been made.
4. Compare and contrast the general ELISA and PCR methods for detecting genetically modified foods. Which method is most commonly used, and why?
5. Design 18 bp PCR primers to amplify (and detect!) the following portion of the cauliflower mosaic virus 35S promoter:  
gtagtgggattgtgctcatcccttacgtcagtg(110 bases)tcaacgatggcctttctttatcgcaatgatggcatttgtaggagc

### Interest Group Questions (only answer the questions for your group)

#### *Entomologists*

Read:

- Hellmich, Richard L., et al., “Monarch larvae sensitivity to *Bacillus thuringiensis*-purified proteins and pollen,” *Proceedings of the National Academy of Sciences*, October 9, 2001; 98 (21): 11925–11930;
- Sears, Mark K., et al., “Impact of *Bt* corn pollen on monarch butterfly populations: A risk assessment,” *Proceedings of the National Academy of Sciences*, October 9, 2001; 98 (21): 11937–11942; and
- Aronson, Arthur, et al., “Why *Bacillus thuringiensis* insecticidal toxins are so effective: unique features of their mode of action,” *FEMS Microbiology Letters*, Feb. 5, 2001; 195(1): 1–8.

Then, answer the following questions:

1. What effect did exposure to *Bt* corn pollen have upon larval survival, leaf consumption, and larval weight?

2. An insect is called an instar when it is between two molts. A newly-hatched insect is called a first-instar or larva. An adult is a final instar. Most caterpillars (butterfly and moth larva) have five or six instars. (To see the different instar stages, go to <http://www.gpnc.org/monarch.htm>.) Does pollen from *Bt* corn affect all instars equally?
3. For *Bt* corn to be toxic to butterflies, what conditions must be true besides the toxicity of the Cry proteins?
4. In one paragraph, describe the biochemical interactions that occur when an insect ingests *Bt* crystal proteins.
5. Describe the three domains of a Cry protein and their putative functions.
6. Next, we will investigate the structure of domain I in greater detail. The PDB code for one of the cry proteins, Cry1Aa, is 1CIY. Open it at <http://firstglance.jmol.org> by entering 1ciy in the box in the middle column and pressing “go.”

- Turn off the spinning by clicking the box next to water in the top left box. Choose “Color N→C Rainbow.” The N terminus is blue, and the C terminus is red. Can you identify the three domains?
- To view just domain I, right click in the box with the protein structure. Choose console from the list, and type “restrict 1-260” (no quotes) and <enter> in the box.
- Center the structure by clicking on the center atom link in the top left box, then click somewhere in the middle of the trace of the domain.

Arginines appear to play a key role in the function of domain I. Let’s examine some of these arginine residues more closely.

- Type “Select arg93, arg224, arg228, arg233” (no spaces between Arg and number; no quotes) in the console then hit <enter>.
- Type “spacefill” and <enter> in the console. The arginines of interest should now be displayed as balls. Mouse over each one to determine the residue number for each. Describe the location of each arginine of interest.

Ex: Arg 93 \_\_\_\_\_ N terminal end of helix 4  
 Arg 224 \_\_\_\_\_  
 Arg 228 \_\_\_\_\_  
 Arg 233 \_\_\_\_\_

Dean et al. (Gene 179 (1996) 111–117) reported the following rates of K<sup>+</sup> current inhibition in gypsy moth (the higher the slope value, the more effective the Cry protein is at destroying the K<sup>+</sup> gradient across the cell membrane) with Cry IAb protein mutated at various positions (R224A means Arg at position 224 is mutated to Ala):

<i>Protein</i>	<i>Slope of short circuit current decay</i>
Wild type (no mutation)	-13.8
R224A	-10.2
R228A	-9.6
R233A	-4.2
No Cry protein	0

- Based on the current data in the table above, which arginine appears to be most important for the function of the cry protein? \_\_\_\_\_
- You will now characterize the interactions of the arginine you selected. Click the “find” link in the top left box. Enter ARGXXX <enter>, where XXX is the number of the arginine you think is most important. The arginine you selected should now be highlighted with yellow halos. Next, select the “contacts” in the top left box. Click the “atoms with Halos” box, then the “show atoms contacting target” link. This command will hide everything except for the atoms contacting the arginine you selected. You may need to zoom in and

center to be able to see everything well. Mouse over the displayed atoms to reveal their identity in the bottom left box. A comment like “Atom: OD1 327 Group: ASP 74” means that you have clicked on the delta oxygen of aspartic acid in position 74. What kind of intermolecular interaction(s) would be disrupted by changing this arginine to an alanine? \_\_\_\_\_

- Write a brief explanation of why mutating your selected arginine to alanine might affect Cry protein function, and design an experiment to confirm your hypothesis.

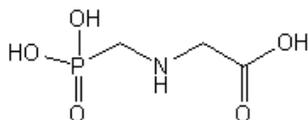
### Farmers

Read:

- “Farm-level effects of adopting genetically engineered crops,” *Economic Issues in Agricultural Biotechnology*, Economic Research Service/USDA, Bulletin AIB-762, p. 10–15;
- Sammons, E. et al., “Reevaluating glyphosate as a transition-state inhibitor of EPSP synthase: identification of an EPSP synthase-EPSP-glyphosate ternary complex,” *Biochemistry*, May 16, 1995; 34(19): 6433–40; and
- Baerson, Scott R., et al., “Glyphosate-resistant goosegrass. Identification of a mutation in the target enzyme 5-enolpyruvylshikimate-3-phosphate synthase,” *Plant Physiology*, July 2002; 129(3): 1265–75.

Then, answer the following questions:

1. What factors have encouraged farmers to use genetically modified plants?
2. Under what conditions do farmers reap an economic benefit from using GM crops?
3. The enzyme affected by Roundup (glyphosate) is 5-enolpyruvylshikimate-3-phosphate synthase, or EPSPS for short. Find the major pathway that utilizes this enzyme. Why is this pathway crucial for plants but not for animals?
4. The crystal structures of EPSPS has been determined with and without one of the substrates, and have PDB codes of 1G6T and 1EPS, respectively. The differences between the two structures can be viewed at <http://www.molmovdb.org/cgi-bin/morph.cgi?ID=073935-730>. Does EPSPS follow the “lock and key” or “induced fit” model of enzyme-substrate binding? Explain.
5. Below is the structure of glyphosate. Draw the structures of the reactants and products for the reaction EPSPS catalyzes. Which substrate has a similar structure to glyphosate? Show the structural parallels between the two structures. Why did this lead to the assumption that glyphosate is a competitive inhibitor?



6. Based on the kinetic data presented in the *Biochemistry* paper, what type of inhibitor is glyphosate for the reverse reaction? Explain your answer.
7. Describe in detail how the sequence of the resistant goosegrass EPSPS enzyme differs from the wildtype (normal) goosegrass EPSPS enzyme.

### Immunologists

Read:

- Hileman, Bette, “What’s hiding in transgenic foods?” *Chemical & Engineering News*, Jan 7, 2002, 20–22;
- Fu, Tong-Jen, et al., “Digestibility of food allergens and nonallergenic proteins in a simulated gastric fluid and simulated intestinal fluid—A comparative study,” *Journal of Agricultural & Food Chemistry*, Nov. 20, 2002;50(24): 7154–60; and
- Kleter, Gijs A., et al., “Screening of transgenic proteins expressed in transgenic food crops for the presence of short amino acid sequences identical to potential, IgE-binding linear epitopes of allergens,” *BMC Structural Biology*, Dec. 12, 2002; 2(1): 8 (<http://www.biomedcentral.com/1472-6807/2/8>).

Then, answer the following questions:

1. What methods are used to predict whether a protein may be an allergen? What are the advantages and disadvantages of each of these models?
2. Describe in detail the conditions used to test the digestibility of proteins and how digestion was evaluated. What criteria should be used in “establishing a globally used standardized assay condition”? Based on the results of this study, what should those criteria be?
3. Computational methods may also help screen for potential allergens. Describe the possible algorithms that could be used for such a screening. What are the advantages and disadvantages of a long and a short reference frame? What would be the advantage of discontinuous epitope searches, and why aren't they currently used?
4. Next, you are going to test the sequence of the Cry protein that is in Starlink corn, Cry9C. You will use the computational methods described by Kleter and Peijnenburg. The sequence is shown below:

```
MADYLQMTDE DYTDSYINPS LSIAGRDAVQ TALTVVGRIL GALGVVPSGQ IVSFYQFLLN
TLWPVNDTAIW EAFMRQVEEL VNQQITEFAR NQALARLQGL GDSFNVYQRS LQNWADRND
TKNLSVVRQA FIALDLDFVN AIPLFAVNGQ QVPLLSVYAQ AVNLHLLLLK DASLFGEGWG
FTQGEISTYY DRQLELTAKY TNYCETWYNT GLDRLRGTNT ESWLRYHQFR REMTLVVLDV
VALFPYYDVR LYPTGSNPQL TREVYTDPIV FNPPANVGLC RRWGTNPYNT FSELENAFIR
PPHLFDRLNS LTISSNRFPV SSNFMDYWSG HTLRRSYLND SAVQEDSYGL ITTTRATINP
GVDGTNRIES TAVDFRSALI GIYGVNRSF VPGGLFNGTT SPANGGCRDL YDTNDELPPD
ESTGSSTHRLS HVTFFSFQTNQ AGSIANAGSV PTYVWTRRDV DLNNTITPNR ITQLPLVKAS
APVSGTTVLK GPGFTGGGIL RRTTNGTFGT LRVTVNSPLTQ QYRLRVRFAS GNFVIRVLRG
GVSIGDVR LG STMNRGQELT YESFFTREFT TTGPFNPPFT FTQAQEILTV NAEGVSTGGE
YYIDRIEIVP VNPAREAAE
```

- a. Search for identical sequences in allergenic proteins:
  - Go to [http://fermi.utmb.edu/SDAP/sdap\\_who.html](http://fermi.utmb.edu/SDAP/sdap_who.html)
  - Enter “Cry9C” in the name box and paste transgenic protein sequence into the search window.
  - Under the “Format” list, set the following parameters:
    - descriptions: 50
    - alignments: 50
  - Click the “Search” button.
  - Identify identical peptide sequences of at least six contiguous amino acids from the results by looking at the alignments. Your sequence is the top, the ones that match are in the middle (+s are related but not identical aa's), and the ones on the bottom are from the allergenic protein. You're looking for 6 continuous letters in the middle line. Record the sequence and location of any matches in our target protein, and the name(s) and accession number(s) of any matches.
  - Verify the identity of the proteins by clicking on the hyperlink in the BLAST result list.
- b. Carry out antigenicity prediction by constructing a hydrophilicity plot with the Hopp and Woods method [A1].
  - Go to the following webpage on the Colorado State University's website:
   
<http://arbl.cvmbs.colostate.edu/molkit/hydrophathy/index.html>
  - Enter the protein sequence in the box and click on the following options:
    - Hopp & Woods
    - Window size: 6
    - Click “plot”.
  - Check if the top position of the highest peak in the plot falls within the identical sequence identified by the BLAST search. Such coincidence indicates a high probability for antigenicity of the identical peptide sequence.

Your answer to this question should include:

- The 6 aa sequence(s) in the protein that matches something in the database and the residue #s of these amino acids
- The name and length of the protein(s) in the database that matched the target protein (notice, the same protein may be in the database more than once)
- Whether the 6 aa sequence(s) might be allergenic, based on the Hopp & Woods data



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