Questions for Serafini et al paper

1. What would you do to separate the nuclear, membrane and cytosolic fractions of a cell?

2. Describe what is the "microsomal" fraction. The netrins are secreted proteins: why do they find them in the microsomal fraction, and not in the extracellular fluid of the embryo?

2. Why can they guess that the netrins are secreted proteins based on their sequence? Does it matter if the sequence that hey are analyzing is the cDNA sequence or the protein sequence that they got from the band that they purified? Why?

3. What experiment can you do to test unambiguously that the netrins are really secreted proteins?

4. why do they bother to put a myc tag into the recombinant netrins that they generate?

5. What could you do to test that inhibiting the netrins in the *in vitro* test that they use, actually blocks the directed growth of the axons?

6. What could you do to test that inhibiting the netrins in *in vivo* (in the animal) actually blocks the directed growth of the axons?

7. why do you think that they go through the trouble of making the recombinant netrins by transfecting COS cells, instead of the much simpler way of growing bacteria that carry a plasmid encoding netrin?

8. Imagine that you are very interested in investigating the existence of an extracellular molecule that could trigger the release of neurotransmitter from neurons.

   8a. Describe in detail the in vitro assay that you would use to test the existence of that molecule.
   8b. how could you test if the molecule is a protein, lipid or sugar?
   8c. how could you identify if the molecule is a low molecular weight compound?
   8d. Describe in detail the strategy that you would follow to identify that molecule.

9. Imagine that for your experiment in 8, you would need 2 different proteins to be present simultaneously to do the job, and that each one of them by themselves will not work. How would you modify your strategy to look for these molecules?

10. Dissecting the brain of a chick embryo takes approximately 5 minutes. If you have to dissect 25,000 of these, and that's all you do in the lab with not interruption, working 60 hours a week, that would take you some 8 months. How could you manage to get other people to help you to finish this task earlier? [Note: this is a serious question. Sometimes the most difficult part of a project is to figure out a way of making it feasible]