BMB170c Problem Set 4: Viruses
Please turn into Peera Tuesday, May 19, 2009

1.) Viruses and evolution (Holmes; Moreira & Lopez-Garcia).

a.) Argue why or why not viruses, plasmids, transposons, retrotransposons, RNA satellites, and spores ought to be considered "living." (10)

There is no right or wrong answer for this. The question here is still a hotly debated topic in the scientific community, and we want to just hear your arguments.

Non-living arguments: see Moreira & Lopez-Garcia, e.g. viruses cannot independently reproduce, no common ancestor (polyphyletic), no structural continuity (no membrane heredity), no metabolism, etc.

Living arguments: virus can evolve, virus has cellular biomolecules, Claverie's definition of "virus factory", etc.

b.) Why can't scientists establish the common ancestor of human RNA viruses farther than a few hundred years ago when there are cases/evidence that they exist (accounts of diseases that are dated past hundred years ago)? (10)

In short, viral lineages turn over very fast.

First, the absolute age of virus cannot be simply taken to be the age of genetic diversity of the sampled viruses. The virus may have diverged from older ancestors. Second, ancient genetic diversity is lost through rapidly evolving genomes of the RNA viruses. Third, RNA viruses usually have short generation time. Fourth, there may be "selection sweeps" that put pressure on the virus to massively undergo genetic changes or wipe out particular lineages of viruses, resulting in artificial short time of shared ancestors.

c.) Between RNA and DNA viruses, which poses a greater pandemic threat? Why? (5)

RNA viruses. They are transmitted across species (swine flu, bird flu, HIV, etc.) and are transmitted horizontally, meaning that they spread very fast in a population. Plus, RNA viruses evolve very rapidly, having much higher evolutionary rates. Also, the virulence was usually high.

2.) Packaging of viral DNA (Petrov & Harvey)

a.) Summarize the main arguments that Petrov & Harvey made regarding different approaches in modeling how DNA gets packaged into the viral capsid. (6)

They prefer the coarse-grain molecular mechanics modeling to the continuum-elastic approximation because no assumption needs to be made regarding the final structure of the packaged DNA or the entropic cost of packaging the DNA. However, this method can handle only small viral genomes.

b.) What are the components that contribute significantly to the free energy in packaging DNA into the capsid? Where does the virus get the energy to overcome these energetic requirements? (6)

Electrostatic repulsion, entropic penalty and elastic deformation (bending energy). The viruses use ATP motor.

c.) Describe at least <u>two</u> experimental approaches that could be used to validate the modeling results. Briefly outline the experiments and discuss the

information you expect to get from each experiment and how it would help validate the modeling studies. (13)

1.) Single molecule pulling/packaging experiments. (attach one end of viral capsid-DNA complex to the bead in the optical trap and let the motor package DNA into the capsid) → can generate force vs length of packaged DNA and give insights into the kinetics of packaging.

2.) Cryo-EM (single particle reconstruction of the viral particle) → see the end conformation of the packaged DNA. Can distinguish between coaxial spools, folded toroids, etc.

3.) AIDS vaccine (Walker & Burton)

a.) What are unique characteristics of HIV-1 that make developing efficient vaccine against HIV extremely difficult? (8)

HIV mutates very fast. Even in an infected individual, there could be enormous diversity. It is very difficult to raise good neutralizing antibodies to something that mutates very rapidly. It infects the cell of the immune system. It evolves mechanisms to evade immune system.

b.) Suppose you are now a new investigator in the HIV vaccine development field. What would be your first <u>two</u> projects? Frame the questions, outline experiments and goals and describe techniques/approaches you plan to employ. Argue why NIH should fund your first grant (these two projects). In other words, convince them why you think these two experiments are the most important steps toward an AIDS vaccine. You may consult the real experienced investigators in the field for some initial ideas, but cite them if you decide to do so. (17)

There are many "right" answers to this question. It depends on your creativity, logical thinking and persuasiveness. Anything that is reasonable and logical would be awarded points.

4.) HIV assembly (Ganser-Pornillos et al.)

a.) Describe in detail, using figures, the structure of the mature HIV virus. Explain what each biophysical technique we studied during the winter term has contributed to that understanding. You may also find the lecture note helpful. (25)

The answers must include what and how we know about the cone shape of the capsid, the spike proteins (gp120/gp41), capsid proteins and their maturation, and the order and organization of a mature HIV. Mostly the techniques include, but not limited to, crystallography of individual domains or proteins, single particle reconstruction by cryoEM, thin section EM, electron crystallography.