Challenges faced by vesicle transport.
1) How is the membrane deformed to form a vesicle?
2) How are the correct cargoes enriched in the vesicle and the proteins that should remain in the donor compartment excluded?

Key to 1 and 2 = coats (protein cages)
- The shape and the chemical properties of the coat proteins help pay the energy cost of deforming the membrane. The coat proteins help neutralize the negative charge on the phospholipids head groups. Some coat proteins have intrinsic curvature, which facilitates the curving of the membrane.
- Coats also help in accomplishing that the correct cargoes get packaged into the vesicles by interacting with proteins that are to be enriched in the vesicle. These interactions may be direct or may be mediated through other proteins.

3) How do vesicles know what compartment to fuse with?
4) What catalyses the fusion process?

Key to 3 and 4 = SNARES
- Specificity in targeting is ensured by pairing between v-SNARES and t-SNARES. Vesicles display v-SNAREs (vesicle SNAREs) on their surface that identify them according to their origin and type of cargo; target membranes display complementary t-SNAREs (target SNAREs) that recognize the v-SNAREs.
- Favorable structural change of SNARE pairing is used to overcome the unfavorable process of membrane fusion (analogous to structural change in gp41 that drives membrane fusion). In the case of SNAREs the pairing of the v-SNARE with the t-SNARE forms a stable bundle of 4 alpha-helices that forces the water molecules from the interface and brings charged lipid head groups together.

5) How does the cell maintain organelles and the plasma membrane at a constant size if vesicles are constantly moving lipids from compartments to the plasma membrane?
6) How does the cell ensure that proteins, which should remain in a compartment do so, even if the process of cargo selection is not perfect?

Key to 5 and 6 = Retrograde Traffic
- Transport in the cell is two-way and not just one-way. This provides an opportunity for proteins that were mis-sorted to be sent back to the correct compartment; retrograde traffic also ensures that the balance of lipids and the relative size of the compartments is maintained.

**Steady State versus Equilibrium**

**Question 7:** Example from lecture: water in a bathtub with a leak from bottom in which water is replaced from a faucet at the same rate as it is leaking out. What if your bathtub had a splash drain that piped the water back into the bathtub? Is this bathtub at steady-state or equilibrium?

**Question 8:** Why doesn’t AZT affect the host cell’s DNA replication?

Two answers: 1. DNA polymerase doesn’t have as high an affinity for AZT compared to RT. 2. DNA polymerase has proofreading capabilities to remove AZT from replicating DNA while RT lacks that function.

**Question 9:** What do CycT and CDK9 normally do?

They enhance the processivity of RNA transcription. However it is unclear how they are recruited to nascent RNAs undergoing transcription. TAR is a sequence near the 5’ end of the nascent HIV RNA transcript. It forms a hairpin loop that TAT recognizes and binds. So during the slow unprocessive transcription, the TAR sequence will be transcribed and made available to TAT. TAT can then bind, recruit Cyclin T and CDK9 which phosphorylate RNA Pol II to enhance the polymerases processivity. Thus TAT increases the amount of HIV RNA transcribed by enhancing processivity of transcription.

**Question 10:** How do you get the first TAT molecule?

Transcription is slow and non-processive, but every so often you will get a full-length transcript. Once that first full-length transcript is made, it will be translated into TAT and REV. The first TAT molecule will subsequently enhance the transcription of additional HIV RNAs – which will encode more TAT, and then the whole process will snowball.

**Question 11:** Based on the position of TAR, do you think HIV has genes encoded on both strands or only one strand of proviral DNA?
Probably only one. The TAR sequence would only be transcribed in one direction from the proviral DNA, and thus transcription in only one direction would be enhanced. This makes it unlikely, but not impossible, that HIV would place genes on the reverse strand.

**Question 12: Prokaryotes do not have nuclei. What are some potential consequences the lack of a nuclear membrane may have upon transcription?**

(Many possible answers): No compartmentalization – the RNA as it is transcribed is immediately exposed to the translation machinery (ribosomes). There is no need to ‘mark’ an RNA for nuclear export.