Viral Structure
- Virion, protein capsid, some have lipid envelope
- Icosahedral, Helical, complex
- Genome: RNA or DNA, single or double stranded, + or – sense, segmented or non-segmented
- Proteins: capsid, attachment proteins, matrix, enzymes

Viral Classification
- Genome and species, Baltimore classification

Infection cycle
- attachment, entry, synthesis, replication, assembly, release

DNA virus replication
- genome replication, transcription, viral assembly in nucleus of host cell (EXCEPT poxviruses!)

RNA virus replication
- requires virally encoded RNA-dependent RNA polymerase
- genome replication in cytoplasm (EXCEPT influenza virus!)
  - + strand RNA: genome transcribed into proteins upon entry into host cell
  - - strand RNA or double strand RNA: has pre-formed polymerase in virion, + strand synthesized upon entry

Retrovirus replication
- ssRNA genome enters with preformed reverse transcriptase (RNA-dependent DNA polymerase), RNA converted to DNA upon entry
- DNA enters nucleus, integrates into host chromosome
- viral DNA transcribed by host RNA polymerase, viral mRNA translated, packaged in cytoplasm

Hepatitis B virus replication
- DNA genome enters nucleus, transcribed to RNA
- RNA transported to cytoplasm, translated, converted to DNA genome by viral reverse transcriptase

Viral release
- Naked viruses lyse cell, enveloped viruses bud from cell

Viral infections: acute vs. persistent

Viral culture usually in tissue culture cells

Diagnosis of viral disease
- Culture, serology, antigen detection, nucleic acid detection, electron microscopy

Viroids: infectious particles consisting only of single RNA molecule, pathogens of plants

Prions: infectious particles consisting only of single protein molecule
- Important pathogens of mammals, causes neurodegenerative “spongiform” encephalopathy
- Infectious protein is misfolded form of normal host protein, can induce misfolding in normal proteins
What is a virus?
A particle consisting of nucleic acid, protein, and in some cases lipids, capable of replication only within cells, utilizing the cellular ribosomes and cellular metabolic energy.

Unlike even the smallest, simplest symbiotic bacteria, even the largest most complex viruses are incapable of generating metabolic energy or synthesizing protein.

• Viruses are important human pathogens producing endemic and epidemic respiratory, gastrointestinal, neurological, and sexually transmitted disease.

• Viruses are the most abundant living entities on the planet and metagenomic studies from randomly sequenced environmental samples have revealed that viral genes constitute the largest part of the genosphere.

• Viruses are major drivers of nutrient and energy cycles on the planet.

General Characteristics of Viruses
- **Virion** (viral particle) is nucleic acid, protein coat
  - Protein coat is **capsid**: protects nucleic acids
    - Carries required enzymes
    - Composed of identical subunits called capsomers
  - Capsid plus nucleic acids called nucleocapsid
  - Enveloped viruses have **lipid bilayer membrane**
  - Matrix protein between nucleocapsid and envelope
  - Naked viruses lack envelope; more resistant to disinfectants

Structural Characteristics of Viruses
- **Genome**
  - DNA or RNA
  - Single Stranded or Double Stranded
  - Positive sense or negative sense
  - Segmented or non-segmented

- **Protein components**
  - Attachment proteins on virion surface recognize receptors on host cells
  - Matrix protein: links the nucleocapsid with the lipid envelope
  - Nucleic acid binding proteins
  - Enzymes: e.g. enzymes necessary for nucleic acid replication

- **Envelope**
  - Lipid bilayer derived from plasma or nuclear membrane of host cell

- **Shapes**
  - Icosahedral, helical, or complex

Why Icosahedral?
- Icosahedron: regular polyhedron with 20 identical equilateral triangular faces, 30 edges and 12 vertices.

- Icosahedral symmetry allows for the lowest-energy configuration of particles interacting isotropically on the surface of a sphere.
Why Helical?

- The simplest way to arrange multiple, identical protein subunits is to use rotational symmetry & to arrange the irregularly shaped proteins around the circumference of a circle to form a disk.
- Multiple disks can then be stacked on top of one another to form a cylinder, with the virus genome coated by the protein shell or contained in the hollow center of the cylinder.

Viral Replication
(We will focus on animal viruses)

- Infection cycle:
  - Receptor recognition and attachment to host cell
  - Viral entry, uncoating, and release of genome
  - Expression of viral proteins
  - Replication of viral nucleic acid
  - Assembly of progeny virions
  - Release of infectious virus from cell

Receptor recognition and attachment to host cell

- Proteins (or glycoproteins) on virion surface recognize and bind to cell surface proteins (glycoproteins)
  - Determines type of cell infected, host species
    - e.g. only human T lymphocytes and macrophage related cells have receptor for HIV
  - Potential target for vaccines, antiviral agents
    - e.g. influenza vaccines induce antibodies which block the interaction the attachment protein, hemagglutinin, with its cellular receptor, sialic acid, on host cell surface glycoproteins.

Viral entry, uncoating, and release of genome

- Entire virion (naked viruses) or nucleocapsid (enveloped viruses) enters the cell
  - Fusion of viral envelope with plasma membrane
  - Endocytosis

DNA Virus Replication

- Transport of genome to nucleus
  - Nucleocapsid, or genome with some associated proteins targeted to nucleus
  - Conversion of ssDNA to dsDNA
  - Transcription of viral DNA by host RNA polymerase
  - Transport of viral mRNA to cytoplasm and translation by host cell ribosomes
  - Replication of viral DNA in nucleus of host cell
  - Combination of host cell, and viral enzymes and proteins
  - Packaging of viral DNA into capsids or nucleocapsids occurs in the nucleus
  - EXCEPTION: Poxxviruses (smallpox virus, vaccinia virus)
    - Replication occurs entirely in cytoplasm
    - Virion contains RNA polymerase for transcription of viral genome
**RNA Virus Replication**
- Tranport of genome to cytoplasm
- If genome is positive sense:
  - + strand acts as mRNA and immediately directs protein synthesis
- If genome is negative sense or double stranded:
  - virion includes preformed RNA-dependent RNA polymerase to generate + sense message using – sense genome as template
- Proteins encoded by virus must include RNA-dependent RNA polymerase
- EXCEPTION: Influenza virus is a negative sense, single stranded RNA virus with RNA replication occurring in the nucleus

**Reverse-Transcribing Viruses**
- Replication involves reverse transcriptase (RNA-dependent DNA polymerase)(RT)
- Retroviruses (e.g. HIV)
  - ssRNA genome enters cytoplasm with pre-formed RT
  - ssRNA converted to dsDNA by RT
  - dsDNA enters nucleus and integrates into host genome
  - viral genes transcribed by host RNA polymerase, yielding viral mRNA and viral genome RNA
  - viral genome, RT, and other proteins packaged in cytoplasm
- Hepatitis B virus
  - partially ds-DNA genome enters nucleus, transcribed to RNA by host RNA polymerase, yielding mRNA, and pre-genomic RNA
  - pre-genomic RNA packaged with viral RT, converted to partially dsDNA genome prior to release

**Release of infectious virus from cell**
- **Naked (non-enveloped) viruses**
  - virion assembly and packaging completed in cytoplasm, and virions released upon disruption of plasma membrane and death of cell
- **Enveloped viruses**
  - viral attachment proteins inserted into plasma membrane and exposed on the surface of the cell
  - viral matrix protein attached to cytoplasmic surface of plasma membrane under attachment proteins
  - nucleocapsid associates with matrix protein
  - virion buds from cell
  - budding process does not involve cell death

**Viral Infections**
- **Acute and Persistent Infections**
  - **Acute**:
    - Rapid onset
    - Short duration
  - **Persistent**:
    - Continue for years or lifetime
    - May or may not have symptoms
    - Some viruses exhibit both (e.g., HIV)

**Cultivating and Quantitating Animal Viruses**
- **Effects of Viral Replication on Cell Cultures**
  - Many viruses cause distinct morphological alterations called **cytopathic effects**
  - Cells may change shape, fuse, detach from surface, lyse, fuse into giant multinuclear cell (**syncytium**), or form **inclusion body** (site of viral replication)

**Diagnosis of Viral Diseases**
- **Clinical diagnosis**
  - based on characteristic signs and symptoms of disease
- **Growth of virus in cell culture**
- **Serology**
  - identification of specific antibodies produced in response to viruses
  - EIA, indirect immunofluorescent microscopy, Western blot
- **Detection of viral antigens using specific antibodies**
  - EIA, direct immunofluorescent microscopy
- **Detection and quantitation of viral nucleic acids**
- **PCR methods**
- **Visualization of virus by electron microscopy**
NOT Viruses

- Viroids: infectious RNA molecules
- Prions: infectious protein molecules

Viroids
- Infectious RNA molecules
- Circular single stranded RNA with extensive base pairing
- Much smaller than RNA genome of viruses
- Do not encode proteins, although do have ribozyme activity
  - Ribozyme: RNA with nuclease activity
- Replicate using ribozyme activity and host enzymes
- Plant pathogens
- Cause disease by base pairing with host mRNA, targeting it for degradation by host enzymes that cleave dsRNA

Prions
- Infectious protein particles with no associated nucleic acid
  - derived from normal host membrane protein, PrP^C, of poorly-understood function
  - Conversion from normal protein to infectious prion, PrP^Sc, involves a conformational change resulting in a more stable, protease resistant structure which forms aggregates and is toxic to neurons.
  - Prions “replicate” by inducing conversion of normal PrP^C to PrP^Sc
- Prion diseases of mammals are slowly progressive neurodegenerative diseases
  - “spongiform encephalopathies” because of sponge-like appearance of brain tissue resulting from neuronal death
- Human disease can result from infectious exposure to prions from another individual or animal OR from spontaneous conversion of PrP^C
  - certain mutations can increase the risk of spontaneous disease

Prion Diseases
- Scrapie: sheep and goats
- Chronic wasting disease: deer and elk
- Bovine spongiform encephalopathy (BSE): cattle and humans, “Mad Cow Disease”
- Kuru: humans
  - now eradicated, was endemic in Papua New Guinea among the Fore tribe
  - Spread by cannibalistic funeral rituals
- Creutzfeld-Jakob disease: humans
  - spontaneous, inherited and infectious forms
  - “Variant Creutzfeldt-Jakob” disease = BSE