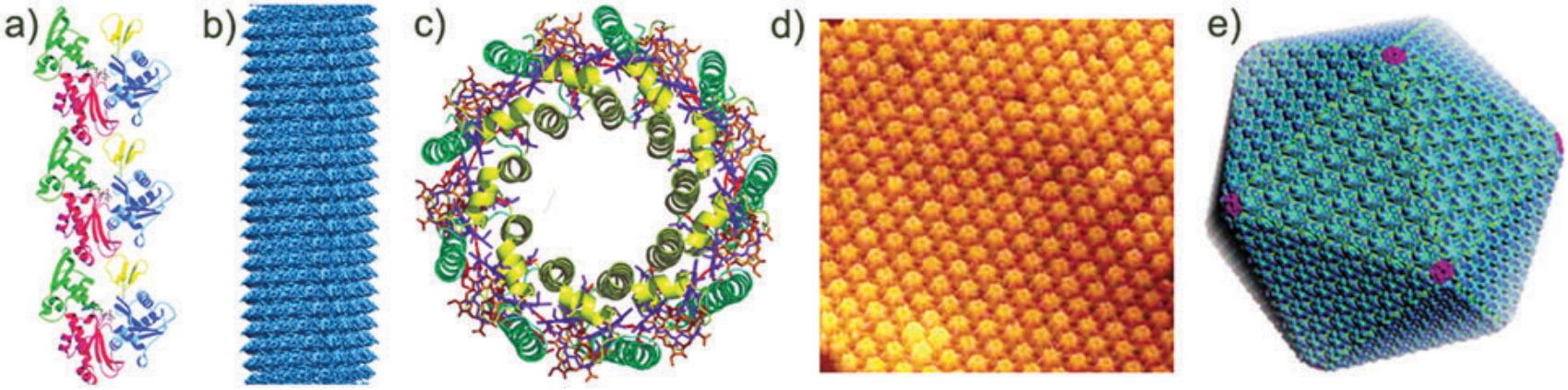
Self assembly



Shape in eukaryotic cells

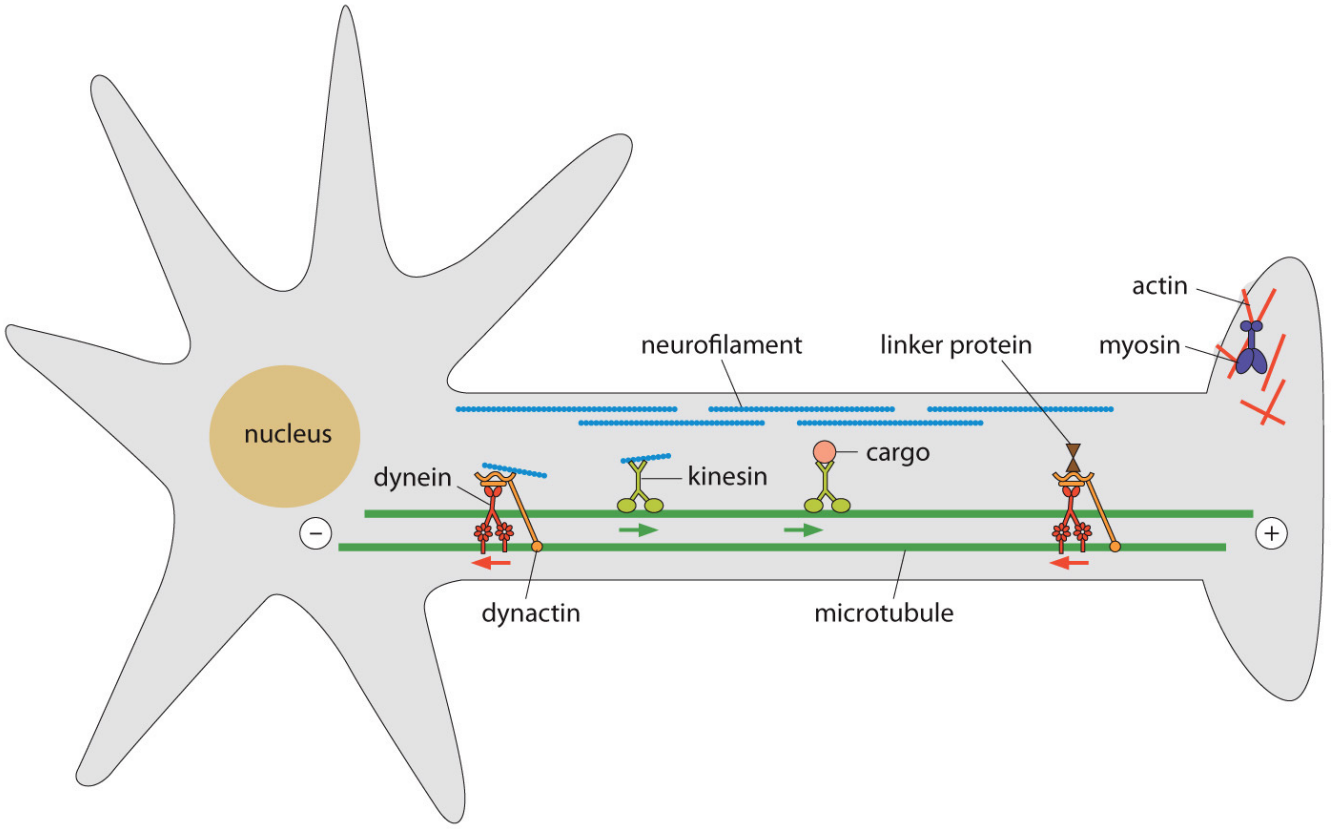


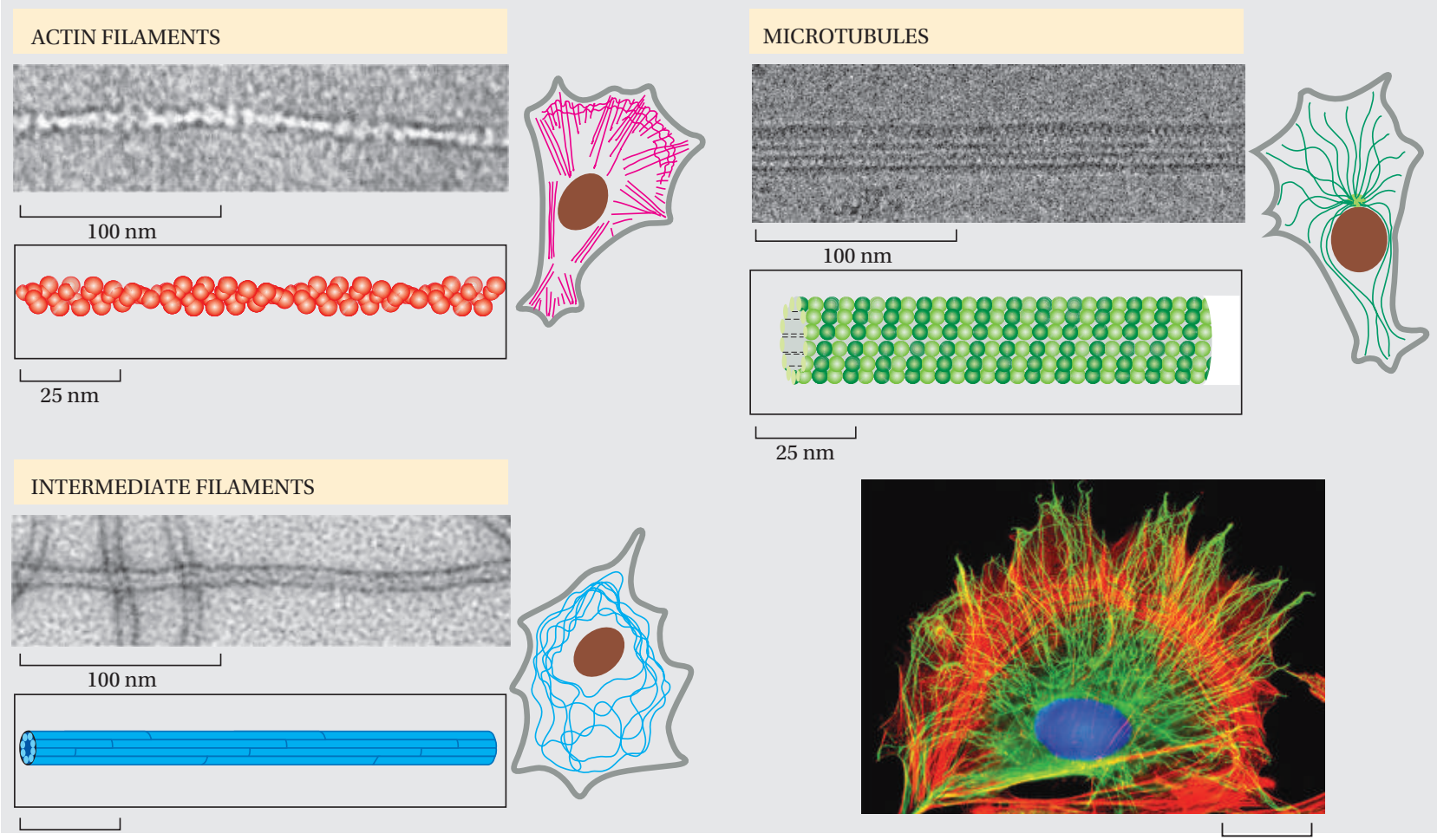
Figure 14.1 Molecular Biology of Assemblies and Machines (© Garland Science 2016)

Shape in eukaryotic cells is provided by the cytoskeleton that consists of actin, tubulin, and intermediate filaments.

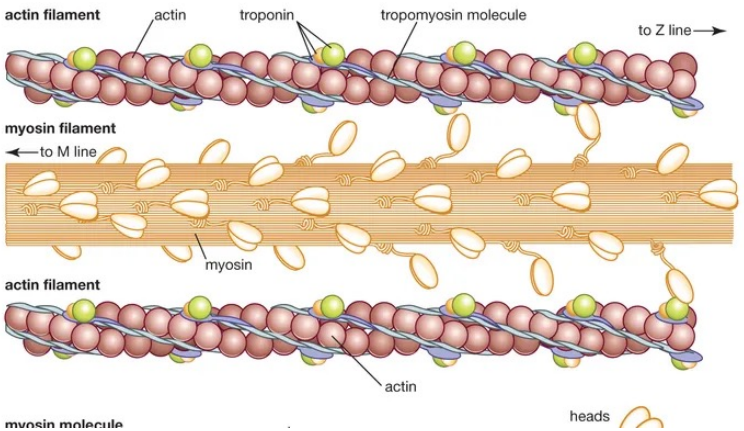
Bacterial cells come in a variety of different shapes, including spheres, rods, spirals, and crescents.

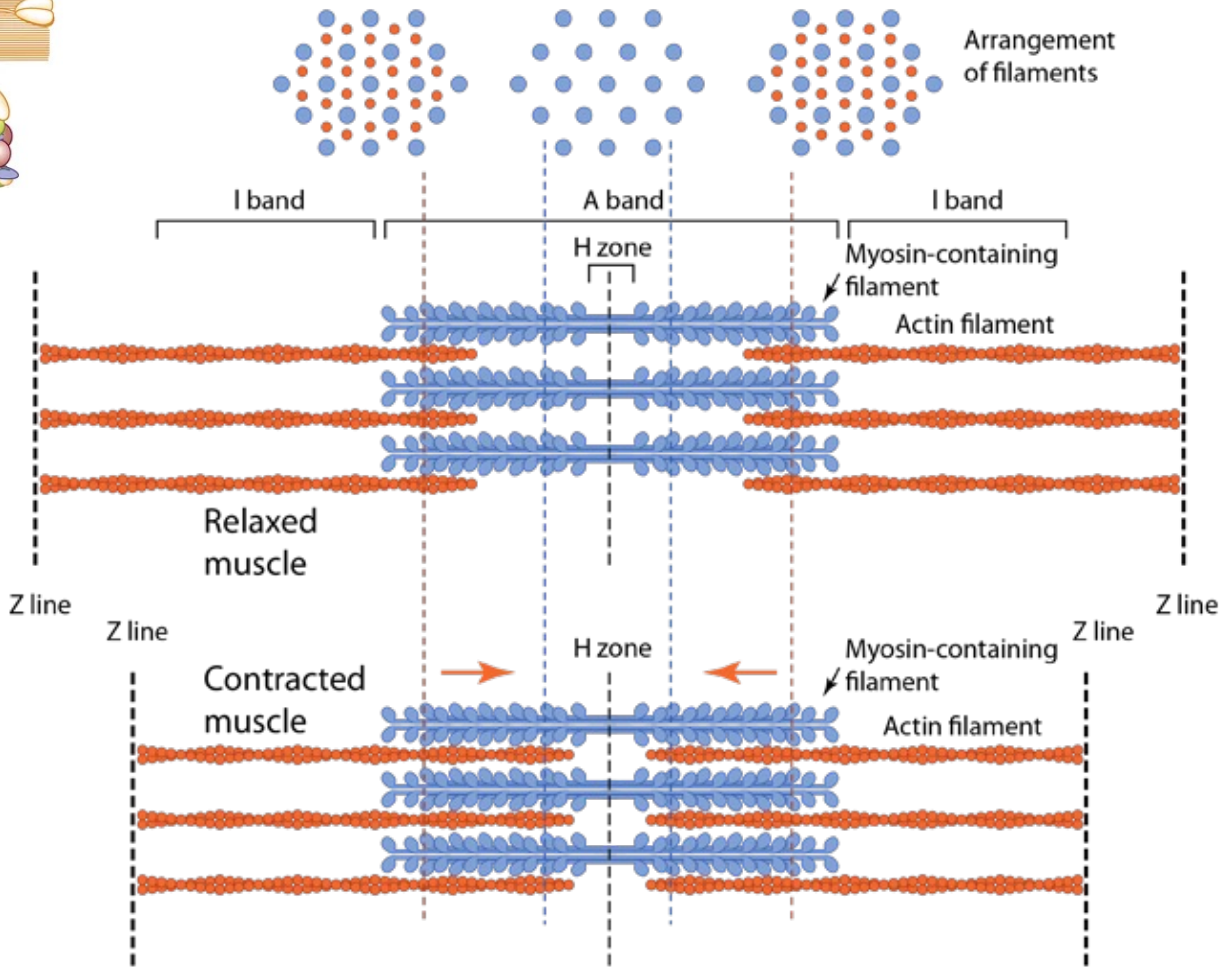
Shape is important for bacterial cells because it plays a role in cell division, helps to maximize the uptake of nutrients, and aids cell movement.

Filaments and microtubules within the cell



Actin


actin filament
actin
troponin
tropomyosin molecule
to Z line
myosin filament
to M line
myosin
actin filament
actin
heads


Arrangement of filaments
I band
A band
I band
H zone
Myosin-containing filament
Actin filament
Relaxed muscle
Z line
Z line
Z line
Z line
H zone
Myosin-containing filament
Contracted muscle
Actin filament

Actin filament

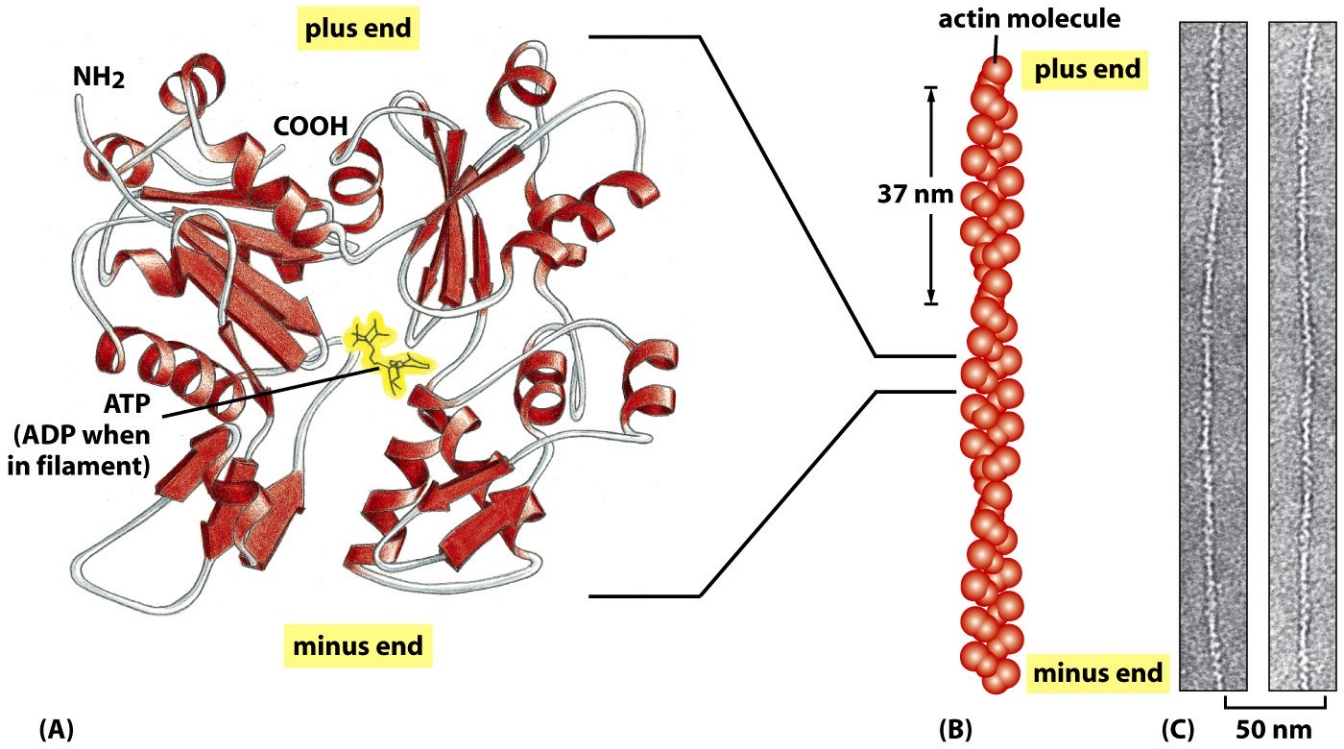
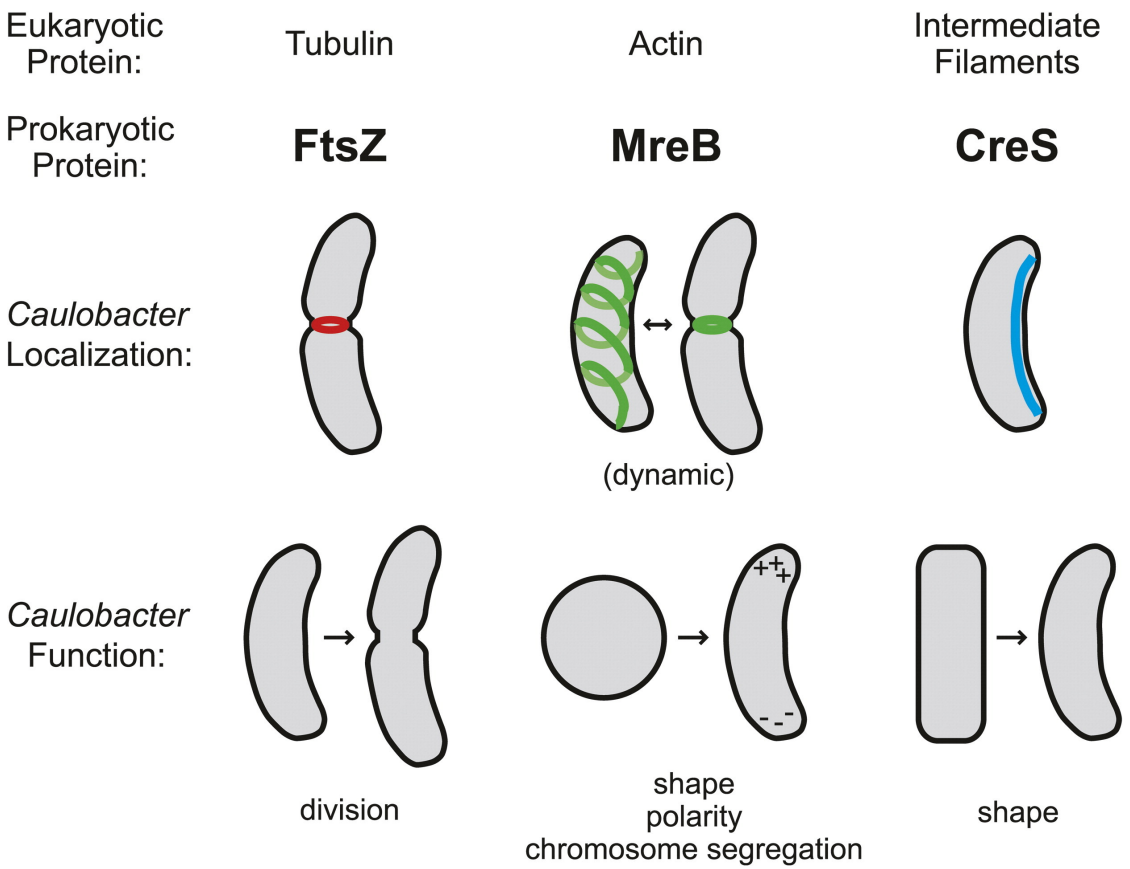


Figure 16-12 *Molecular Biology of the Cell* (© Garland Science 2008)

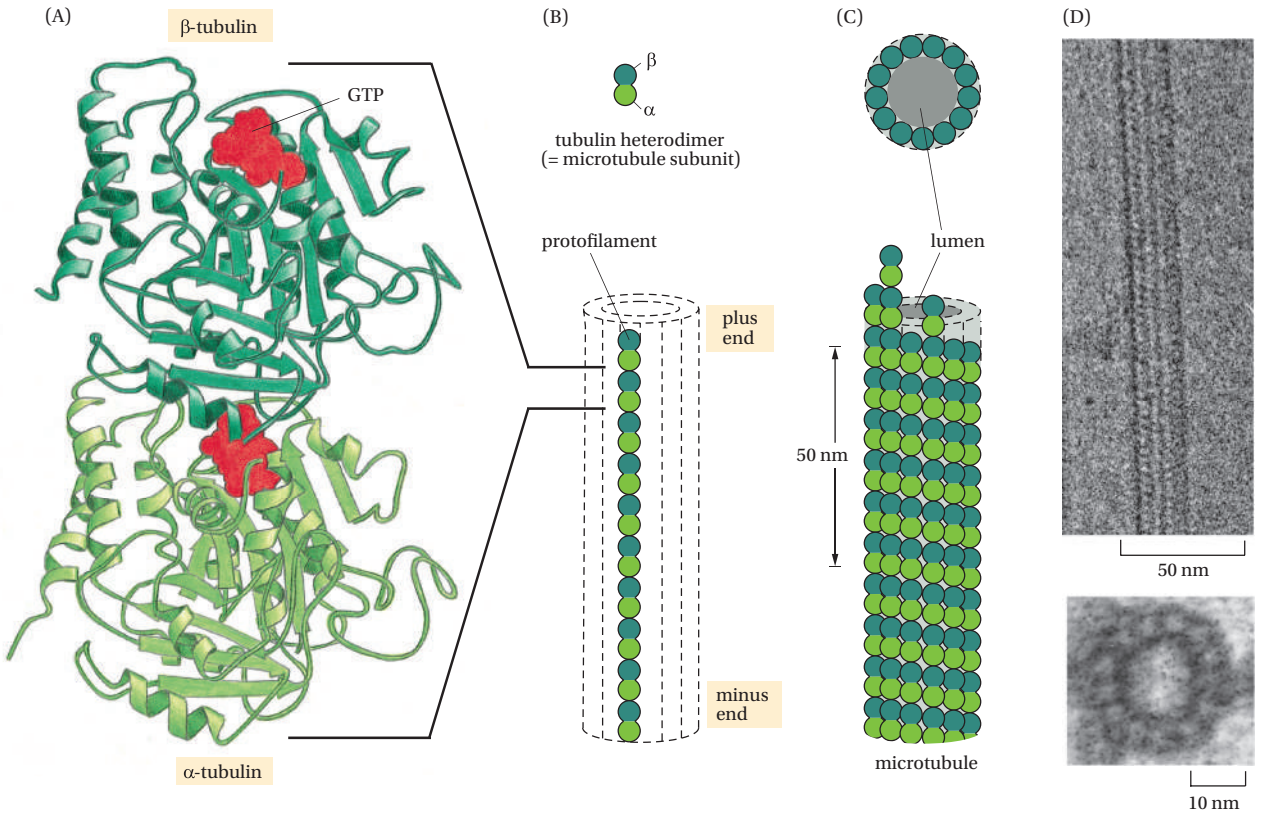
Procaryotic actin

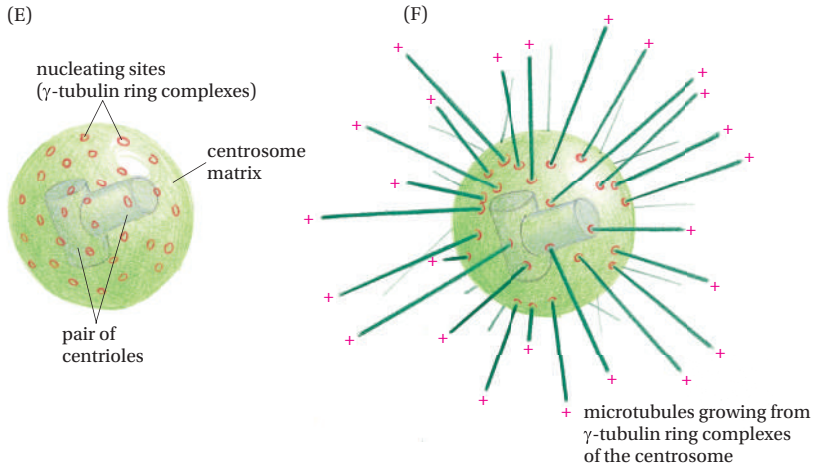
Prokaryotes also have a dynamic, filamentous network of proteins, which are homologous to the eukaryotic cytoskeletal elements.

In non-spherical bacteria, the actin homologue MreB is essential for shape maintenance as depletion of MreB through genetic knockouts or MreB-targeted drug treatment results in misshapen cells that eventually lyse

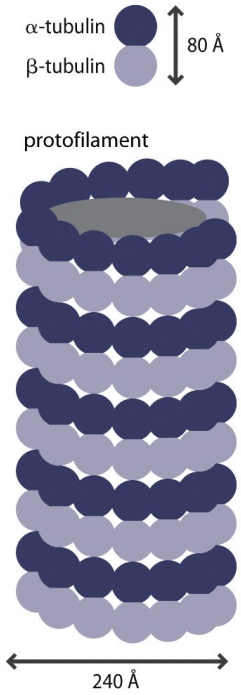


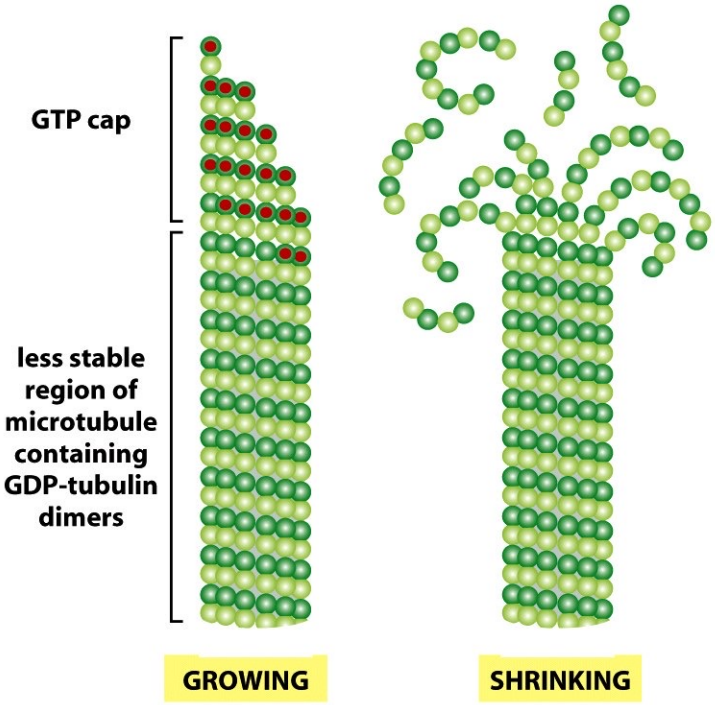
microtubules





Dynamic instability





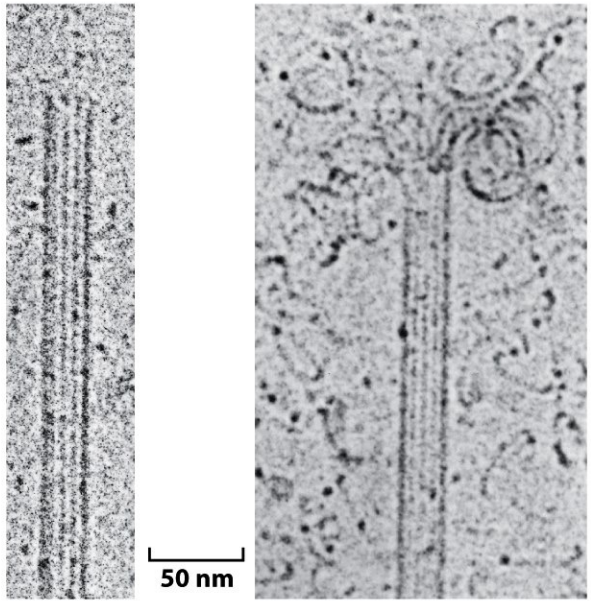


Figure 7.27 How Proteins Work (©2012 Garland Science)

Tubulin interacting proteins

TABLE 7.2 Proteins that interact with tubulin/microtubules

Protein	Function
γ-TuRC	Initiates filament formation
MAP, XMAP215	Stabilizes filaments
Tau, MAP-2	Cross-links filaments in parallel rows
Stathmin, kinesin 13, katanin	Cuts or depolymerizes filaments
+TIP, plectin	Links filament to other proteins

Table 7.2 How Proteins Work (©2012 Garland Science)

Fiber growth

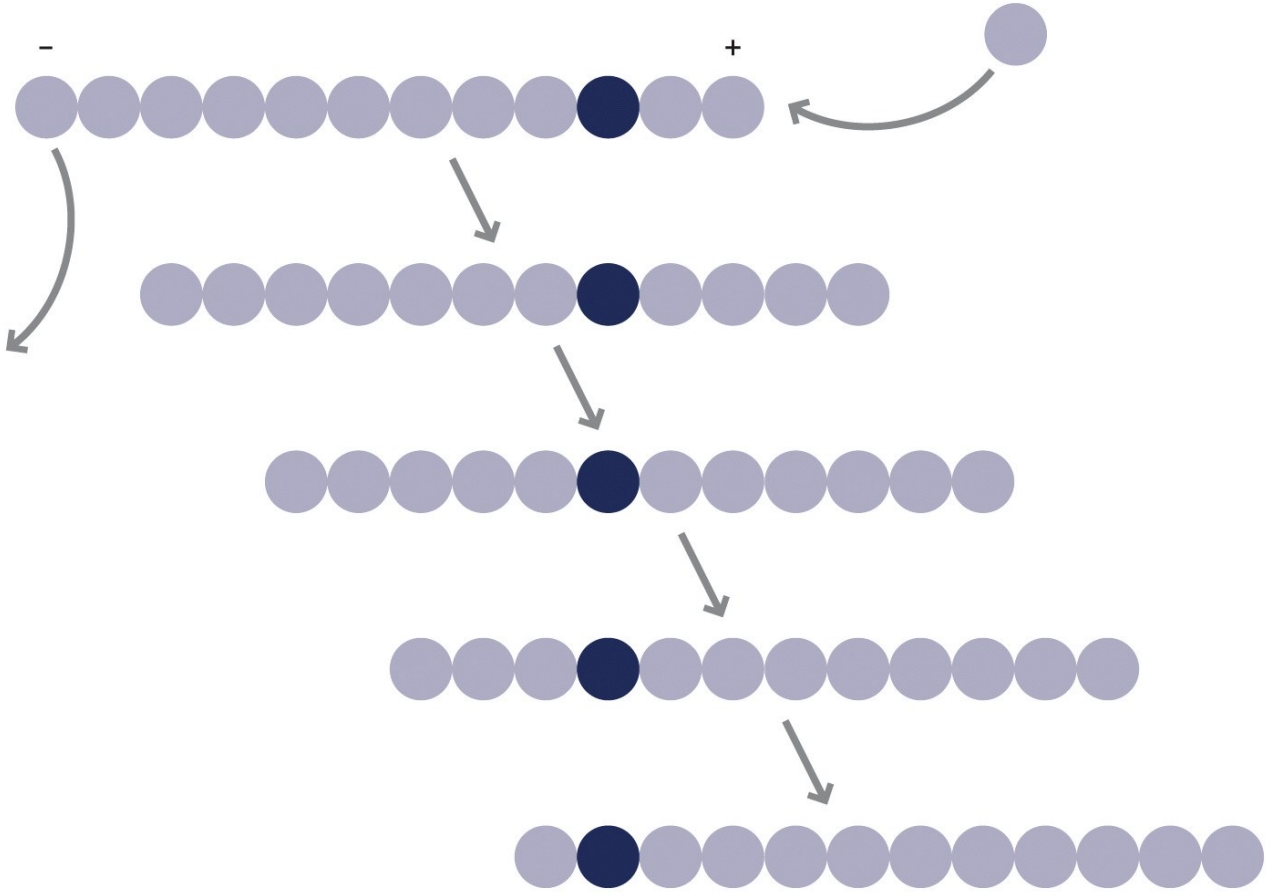
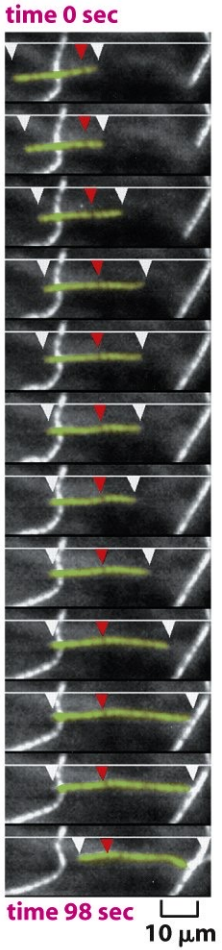
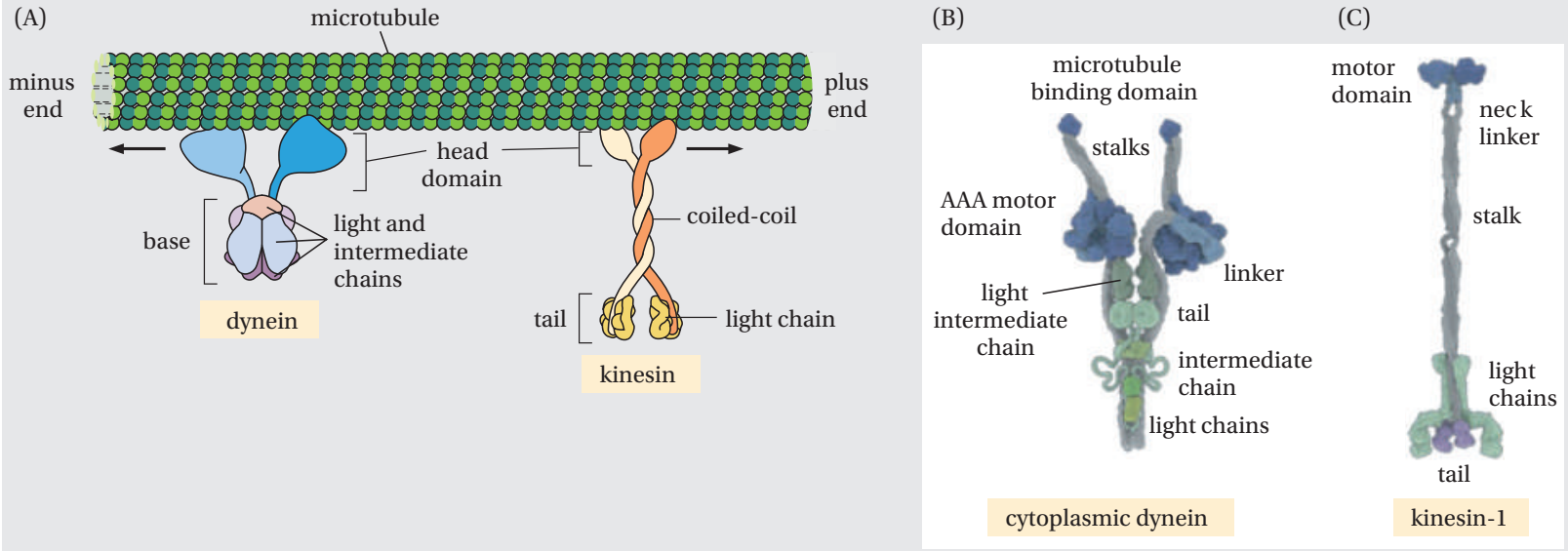


Figure 7.30 How Proteins Work (©2012 Garland Science)



kinesins and dyneins



Drugs

Table 16–2 Drugs That Affect Actin Filaments and Microtubules

ACTIN-SPECIFIC DRUGS	
Phalloidin	binds and stabilizes filaments
Cytochalasin	caps filament plus ends
Swinholide	severs filaments
Latrunculin	binds subunits and prevents their polymerization
MICROTUBULE-SPECIFIC DRUGS	
Taxol	binds and stabilizes microtubules
Colchicine, colcemid	binds subunits and prevents their polymerization
Vinblastine, vincristine	binds subunits and prevents their polymerization
Nocodazole	binds subunits and prevents their polymerization

Fluorescence microscopy

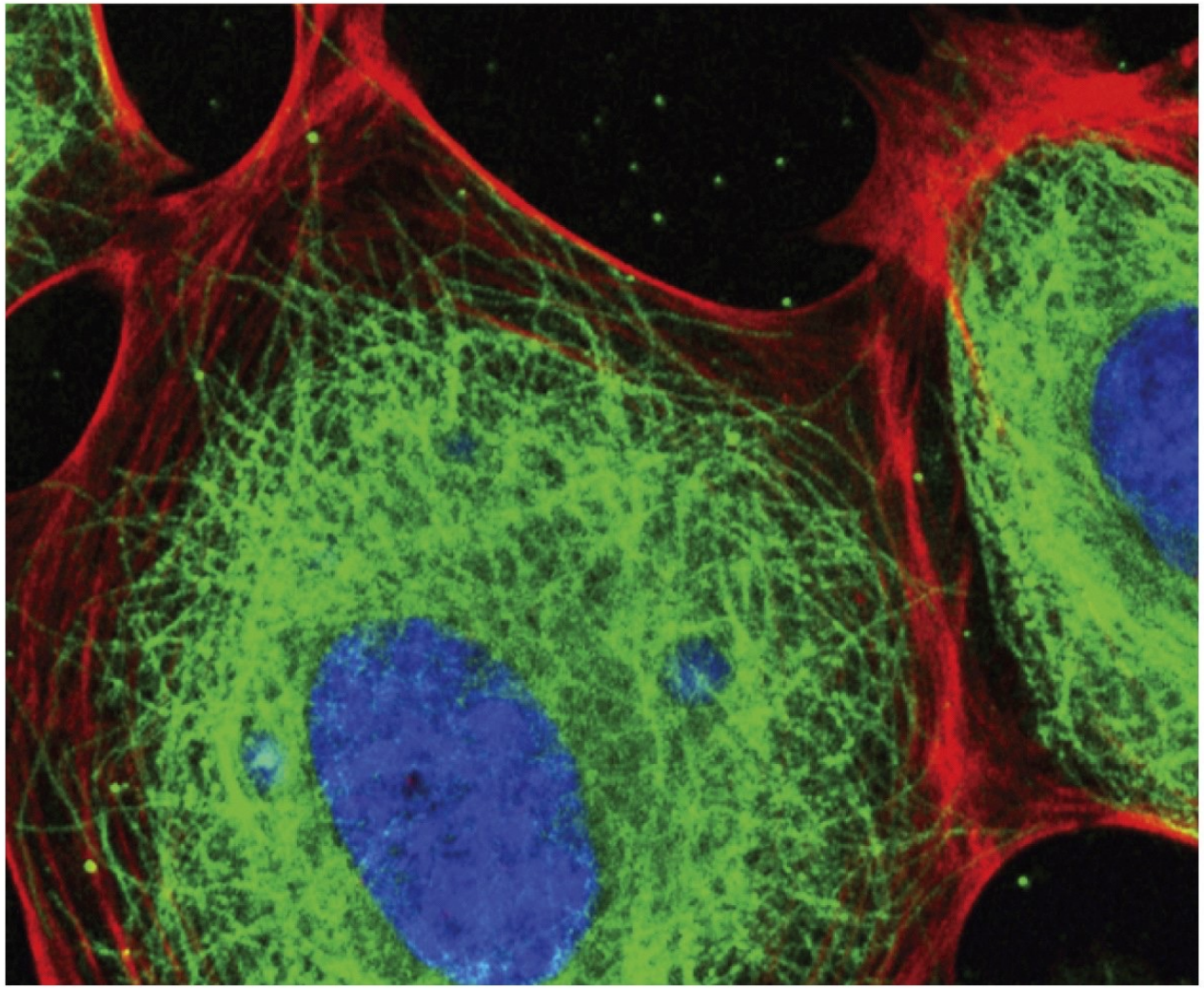
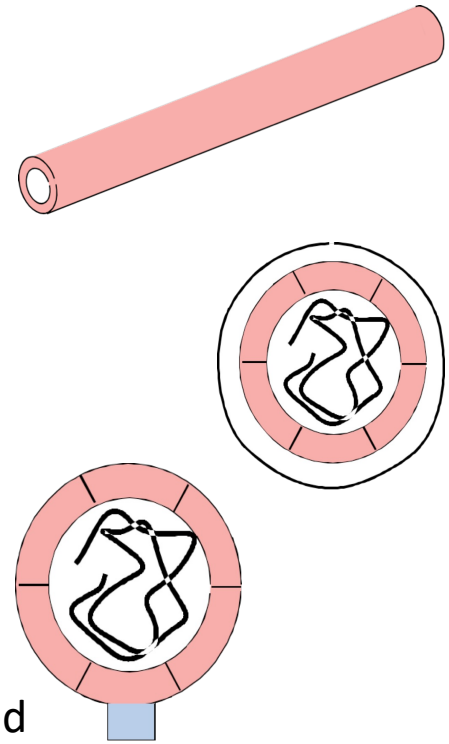


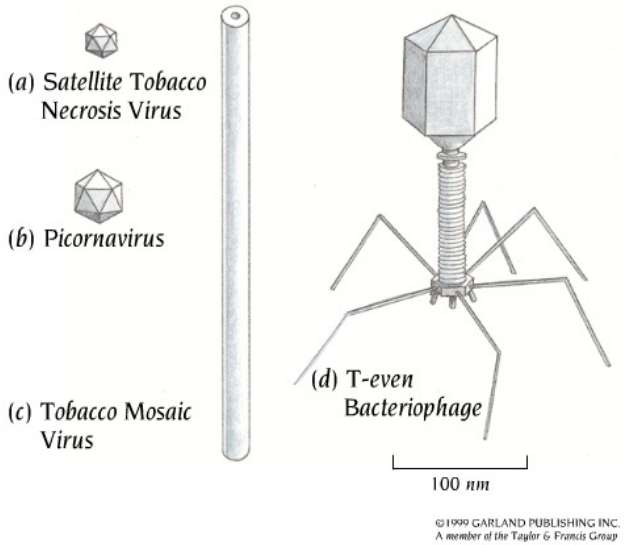
Figure 7.33 How Proteins Work (©2012 Garland Science)

Virus Structure

Virus Structure

- Size
  - 17 nm – 3000 nm diameter
- Basic shape
  - Rod-like
  - “Spherical”
- Protective Shell - Capsid
  - Made of many identical protein subunits
  - Symmetrically organized
  - 50% of weight
  - Enveloped or non-enveloped
- Genomic material
  - DNA or RNA
  - Single- or double-stranded





Virus Structure

- Virus capsids function in:
  - Packaging and protecting nucleic acid
  - Host cell recognition
    - Protein on coat or envelope “feels” or “recognizes” host cell receptors
  - Genomic material delivery
    - Enveloped: cell fusion event
    - Non-enveloped: more complex strategies & specialized structures

Viruses

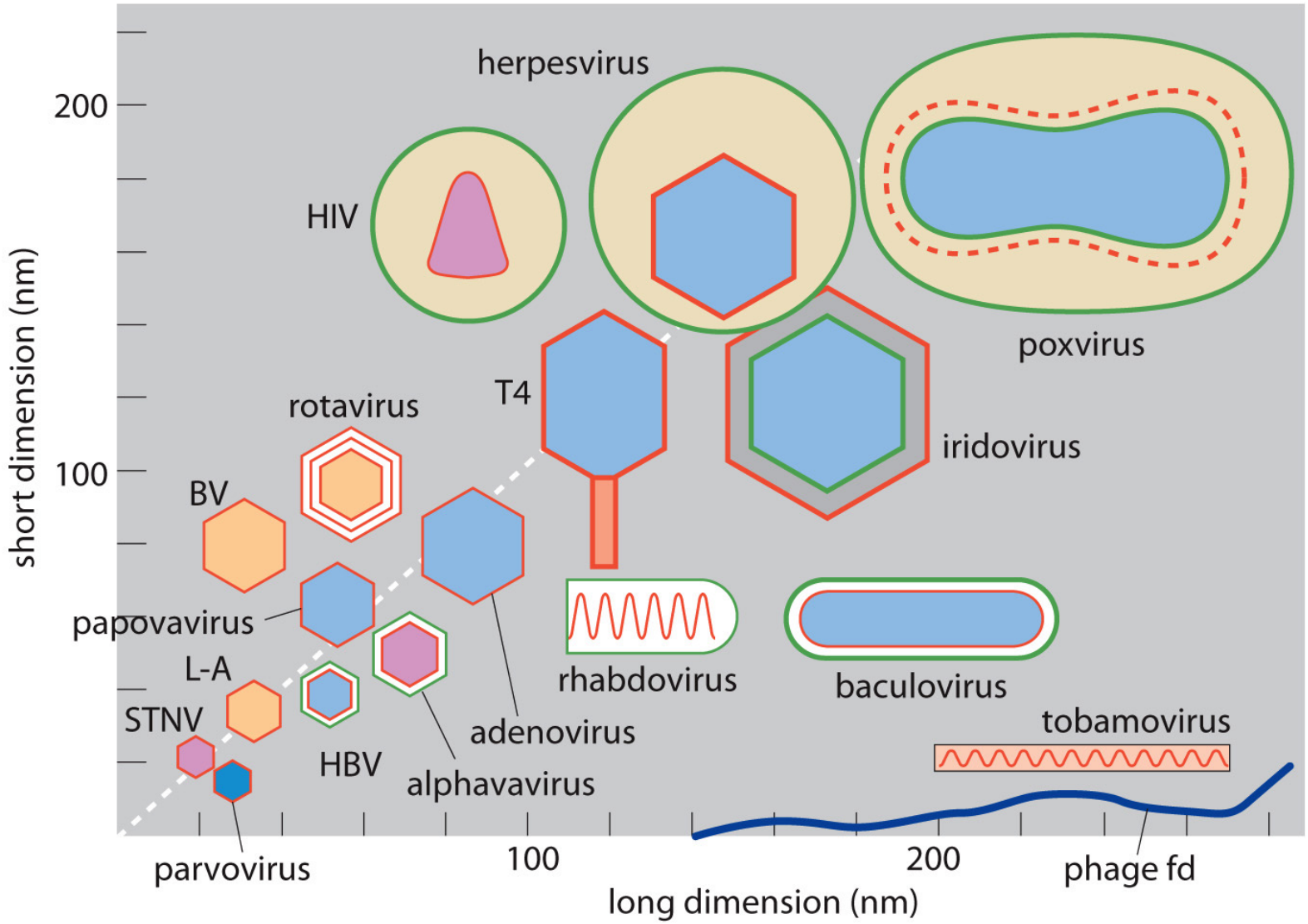


Figure 8.4 Molecular Biology of Assemblies and Machines (© Garland Science 2016)

assembly pathways

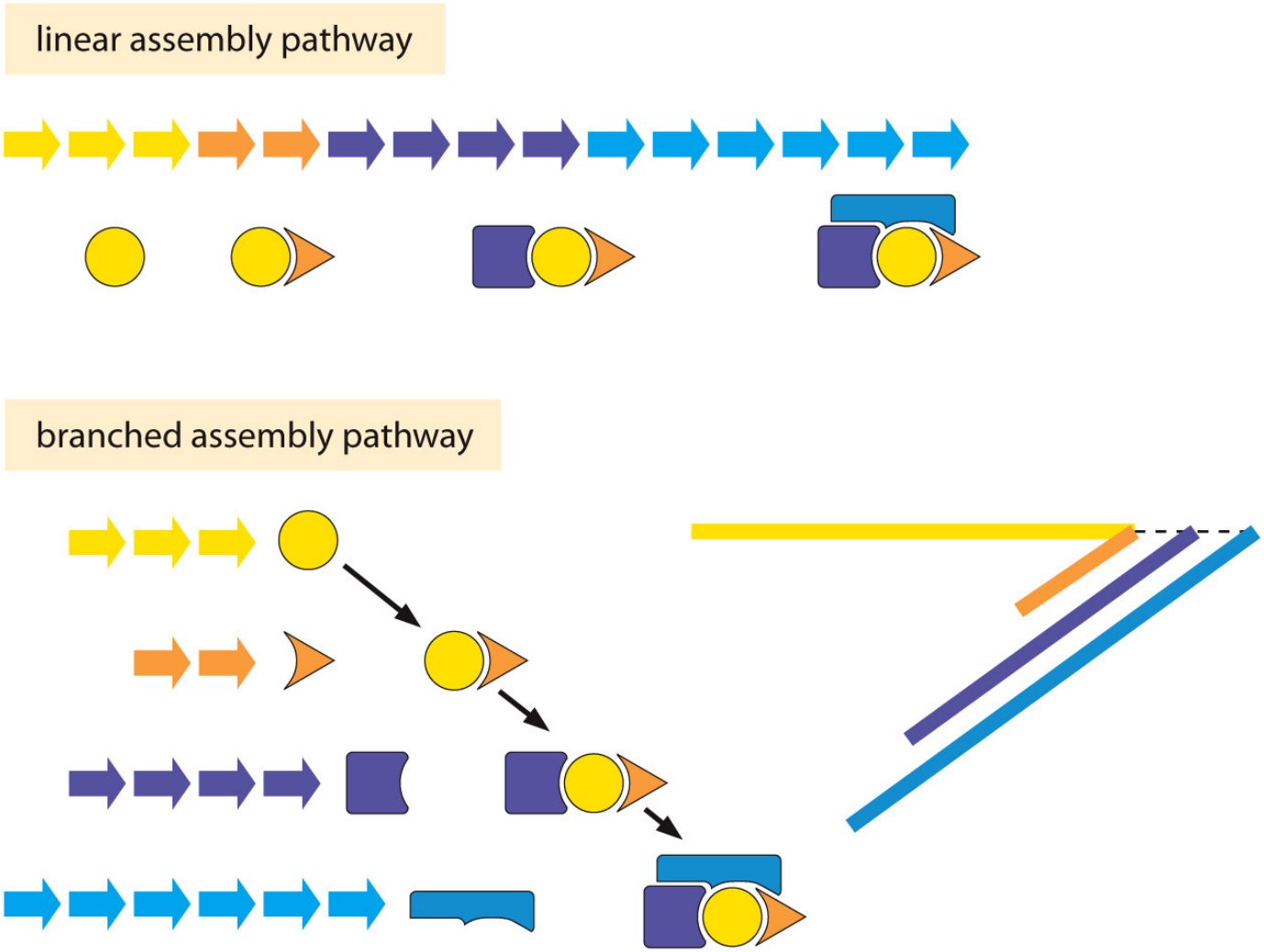


Figure 8.2a Molecular Biology of Assemblies and Machines (© Garland Science 2016)

bacteriophage T4

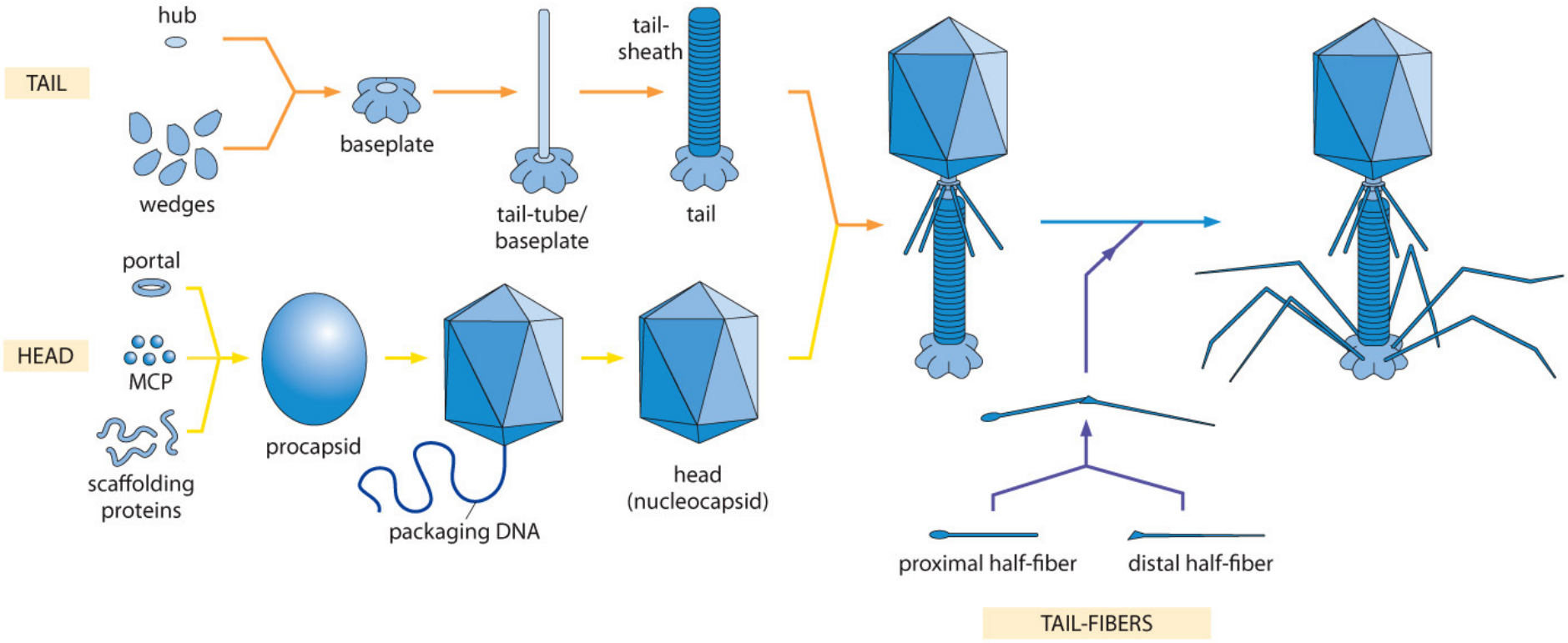
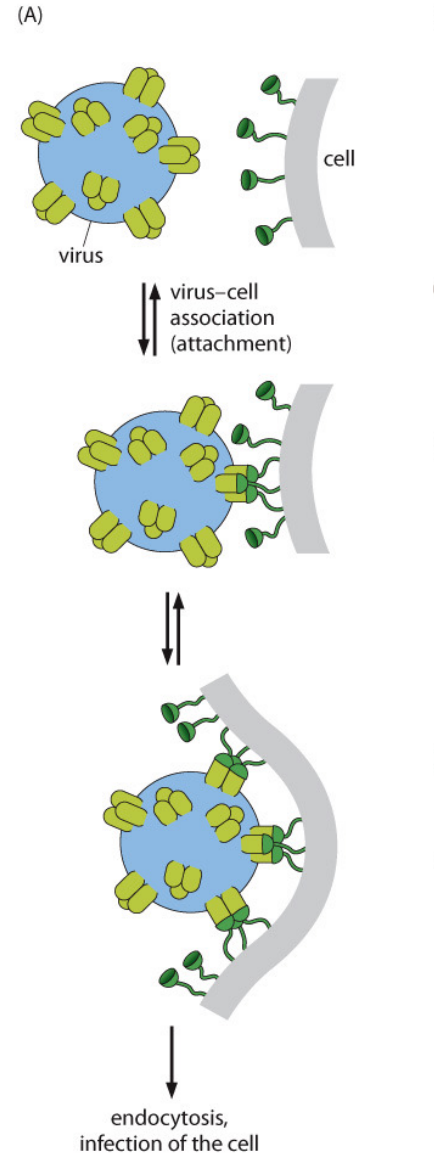


Figure 8.2b Molecular Biology of Assemblies and Machines (© Garland Science 2016)

recognition



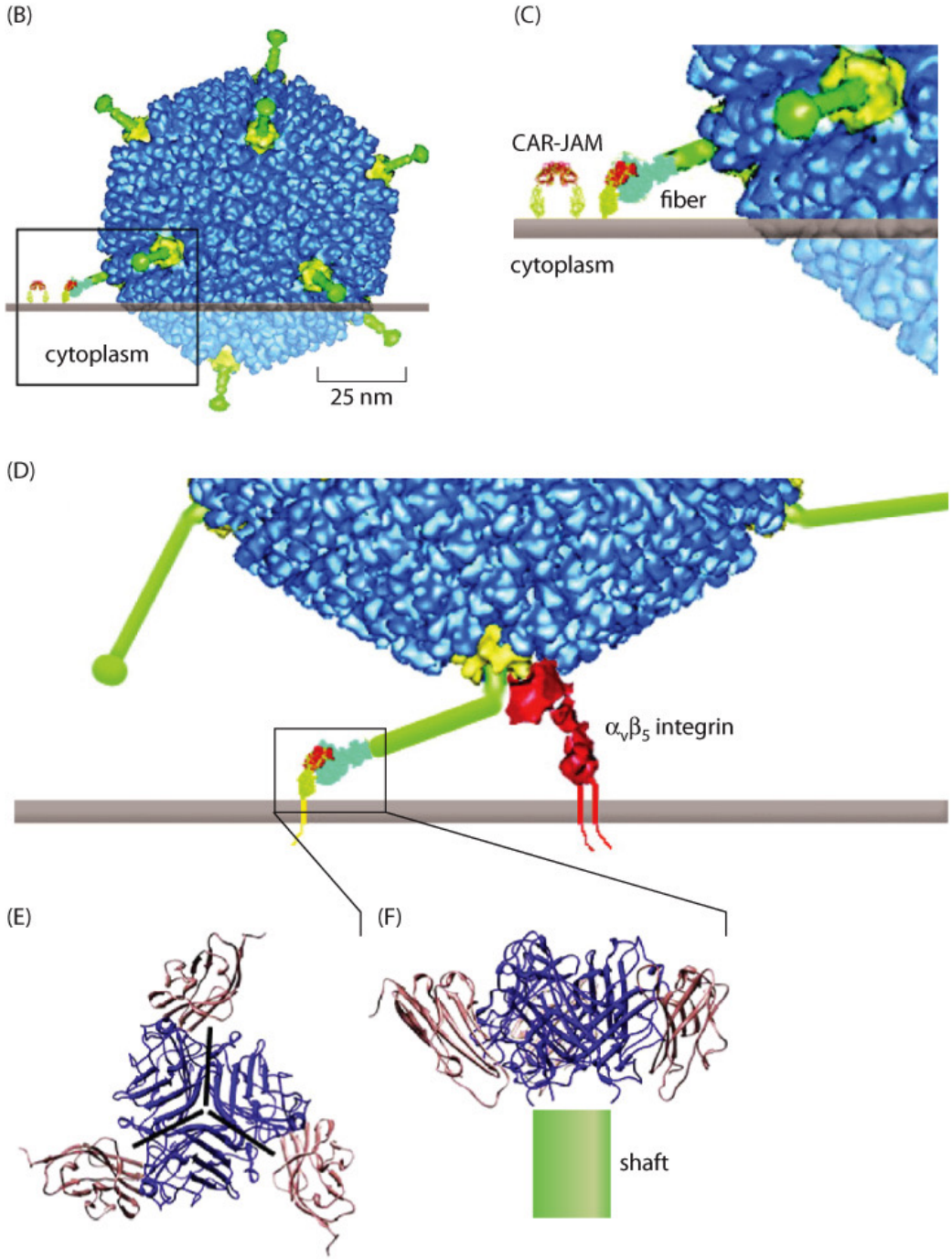
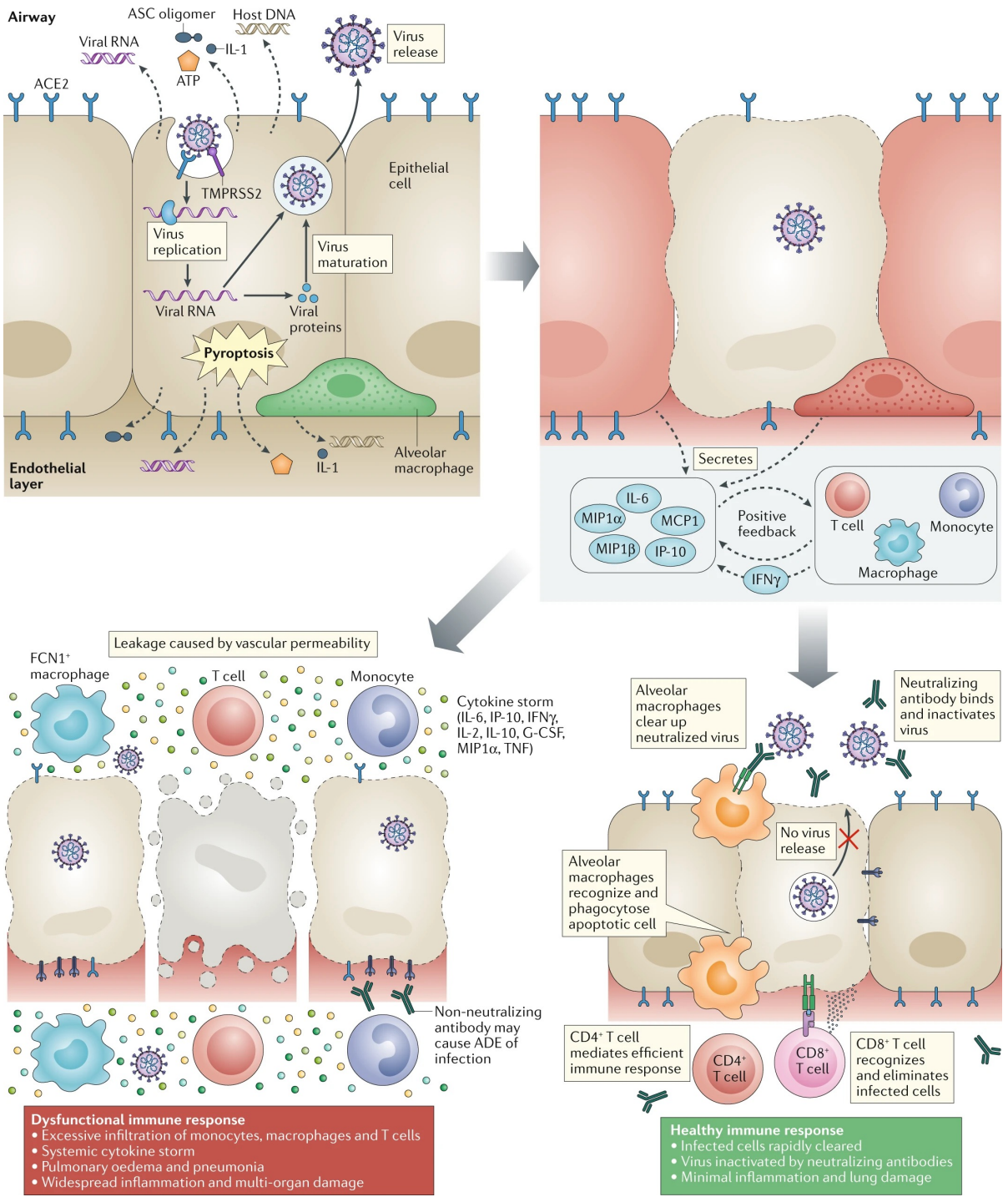


Figure 8.3 Molecular Biology of Assemblies and M

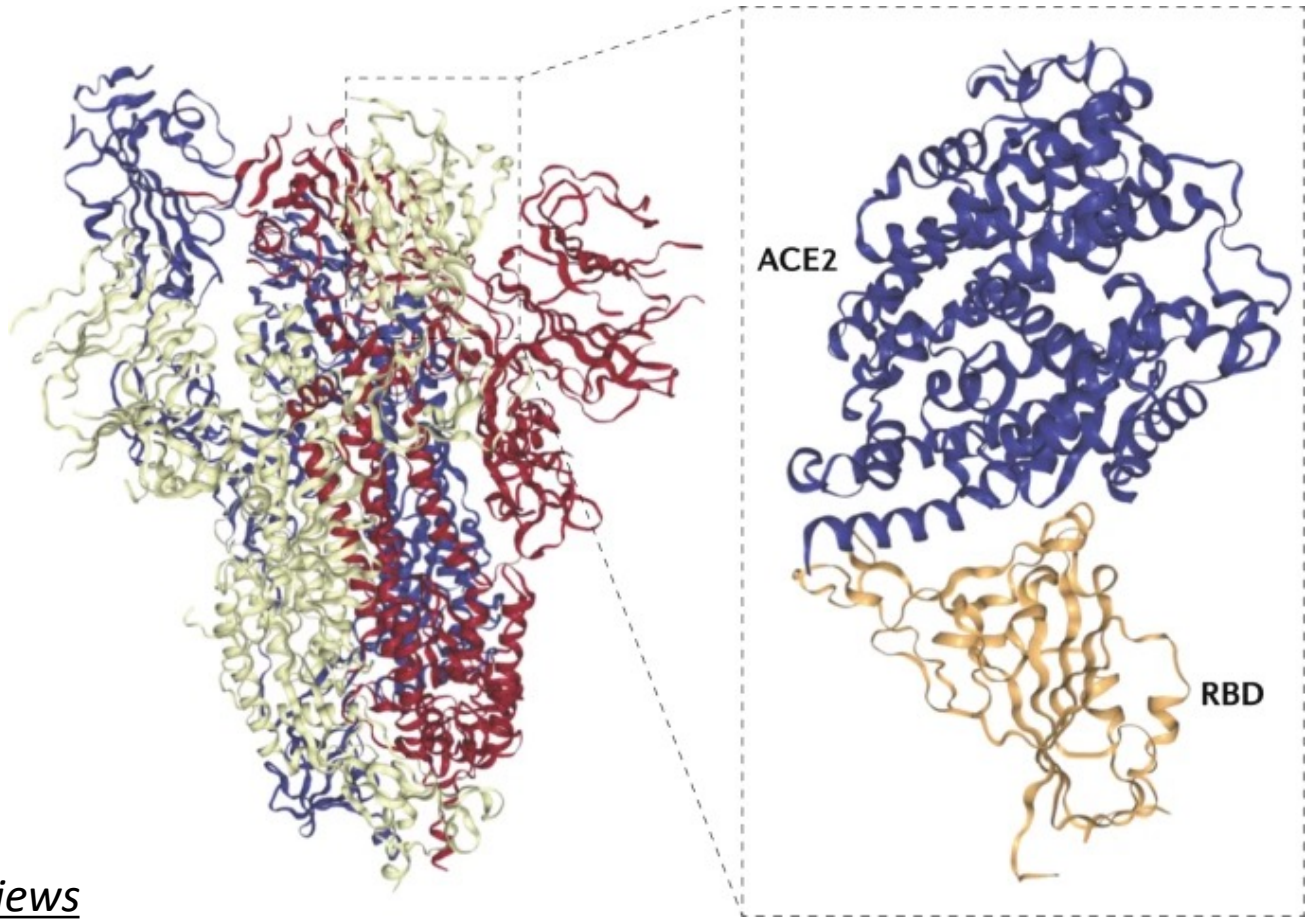
d Machines (© Garland Science 2016)

recognition



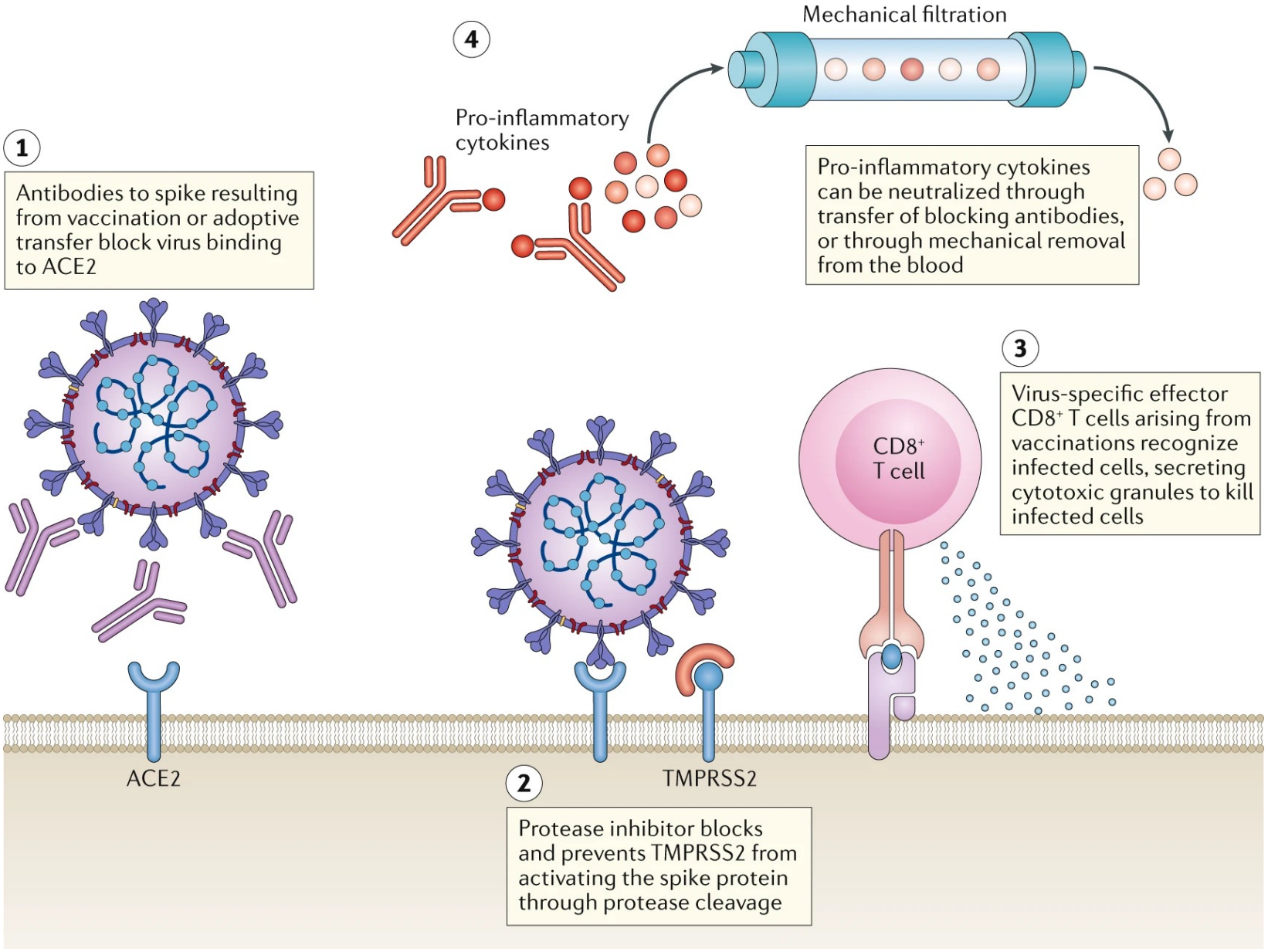
Nature Reviews Immunology volume 20, pages 363–374 (2020)

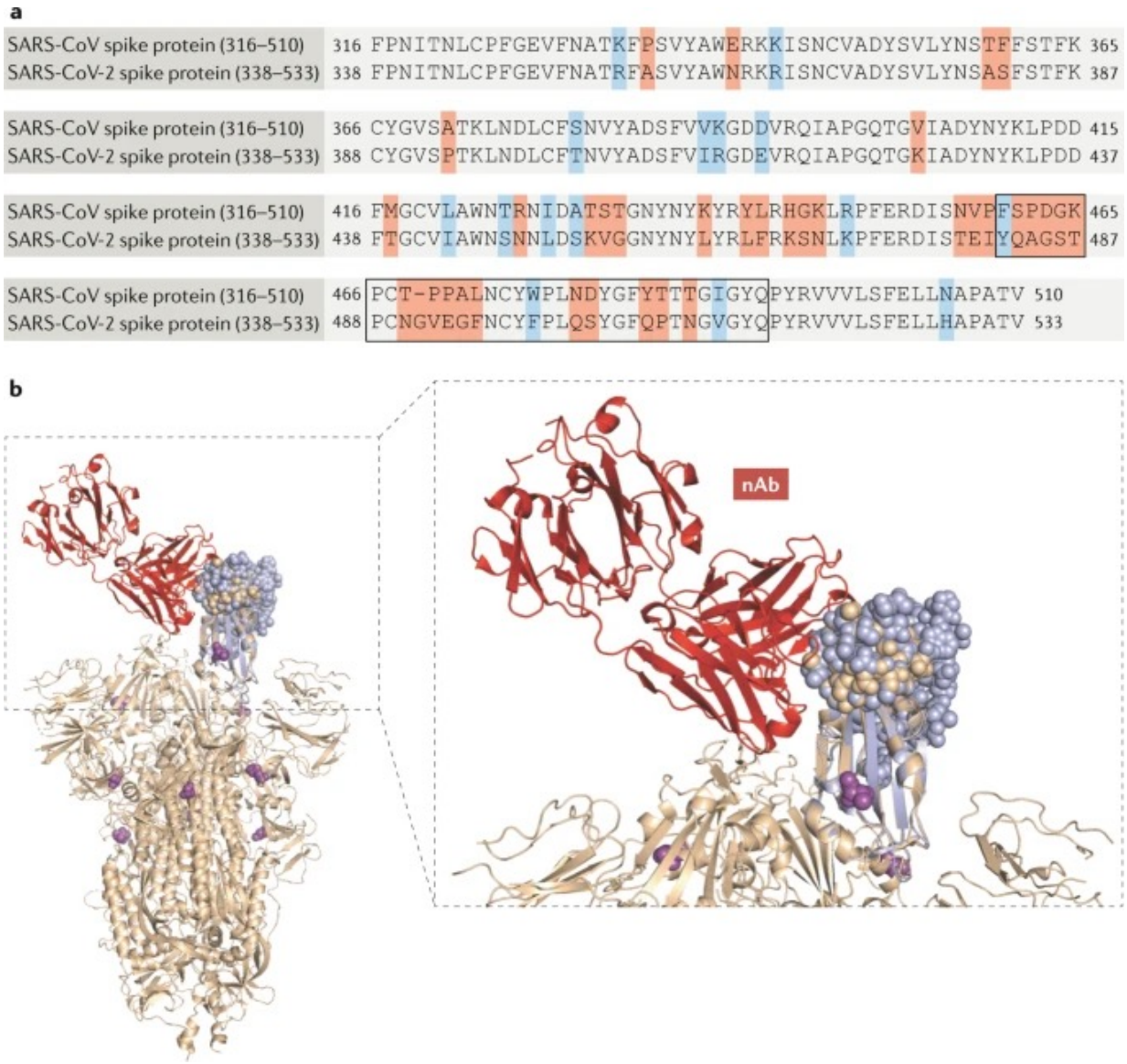
The structure of the trimeric spike protein of SARS-CoV-2.



Nature Reviews Immunology volume 20, pages 363–374 (2020)

Potential therapeutic approaches





<u>*Nature Reviews Immunology*</u> volume 20, pages 363–374 (2020)

History

- In 1953, Crick & Watson proposed … principles of virus structure
  - Key insight:
    - Limited volume of virion capsid => nucleic acid sufficient to code for only a few sorts of proteins of limited size
  - Conclusion:
    - Identical subunits in identical environments
    - Icosahedral, dodecahedral symmetry

- In 50's & 60's Klug and others confirmed that several (unrelated) "spherical" viruses had icosahedral symmetry
  - (Used negative staining & electron microscopy)
- Conclusion:
  - Icosahedral symmetry is preferred in virus structure

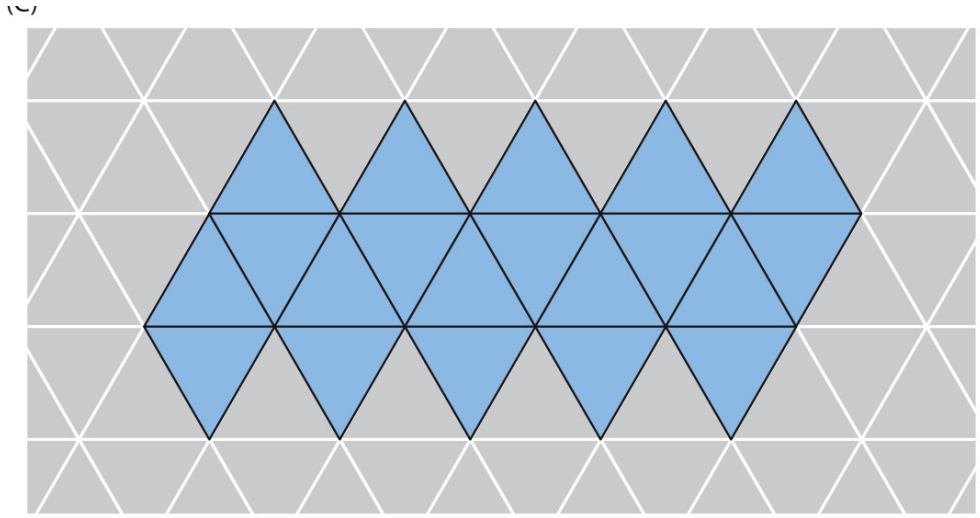
Icosahedral Symmetry

12 vertices

20 faces (equilateral triangles)

5-3-2 symmetry axes

60 identical* subunits
in identical environments
can form icosahedral shell
* asymmetric



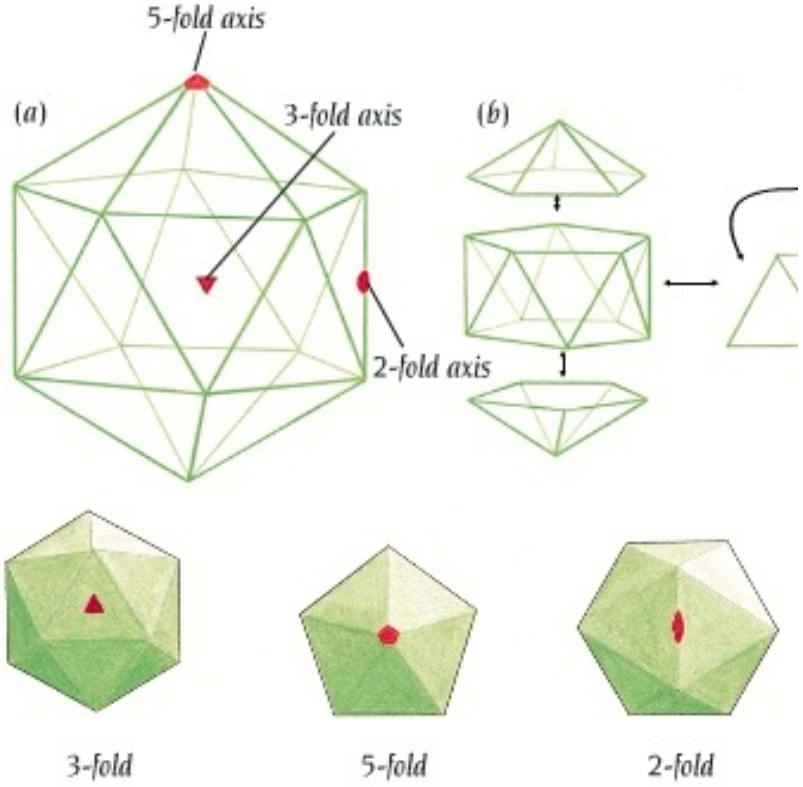


Figure 8.6 Molecular Biology of Assemblies and Machines (© Garland Science 2016)

But …

- Clear evolutionary pressure to make larger capsid
  - Using larger subunits helps very little
  - Using more subunits helps a lot

- Not possible to form icosahedral shell (of identical units in identical environments) with more than 60 subunits

- Viruses with more than 60 subunits were observed

- In 1962, Caspar & Klug proposed the theory of "quasi-equivalence"

  - Not all protein subunits are equivalent
    - "Identical" subunits in slightly different environments

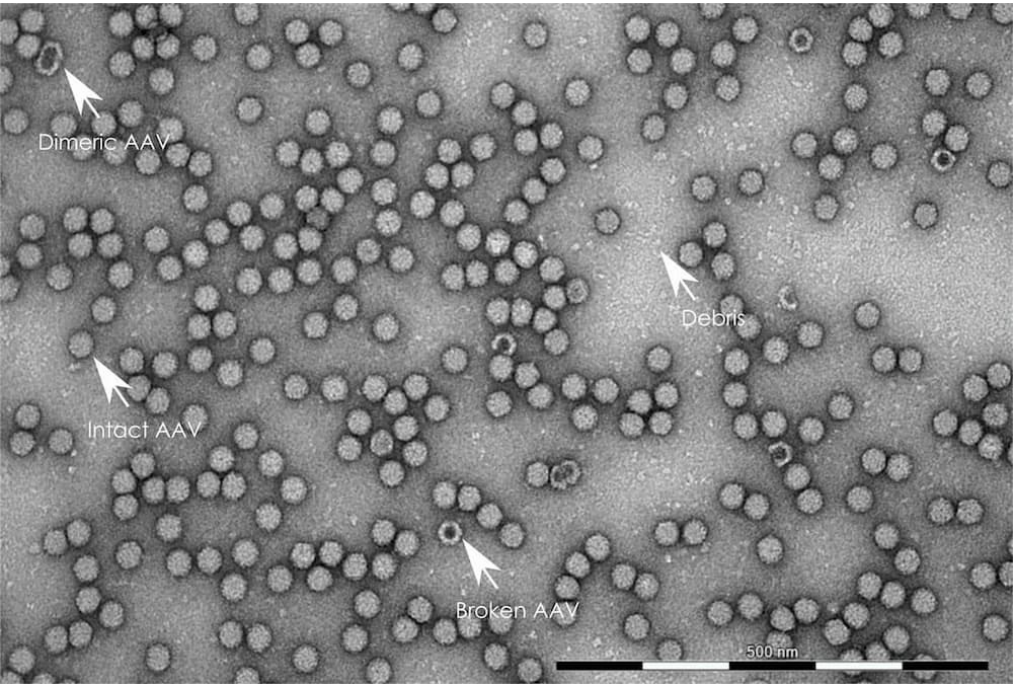
  - Only certain numbers of subunits will can be packed into closed regular lattice.

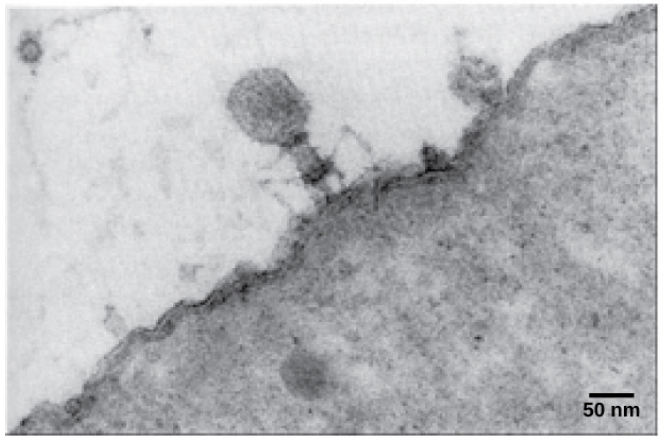
X-ray Crystallography of Viruses

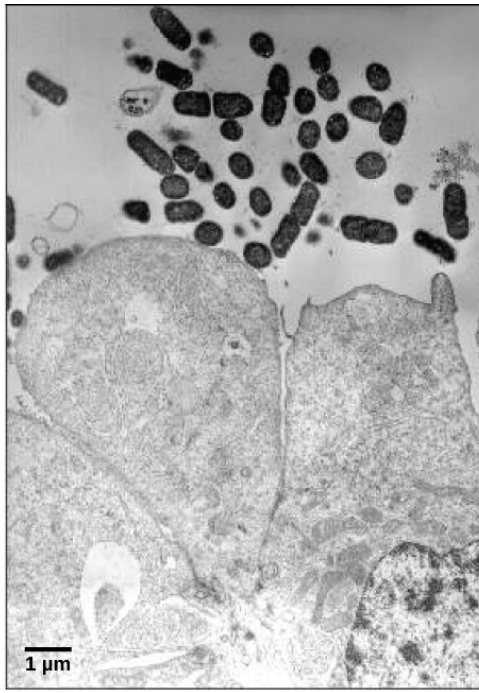
- Symmetry of protein shells makes them uniquely well-suited to crystallographic methods

- Viruses are the largest assemblies of biological macromolecules whose structures have been determined at high resolution

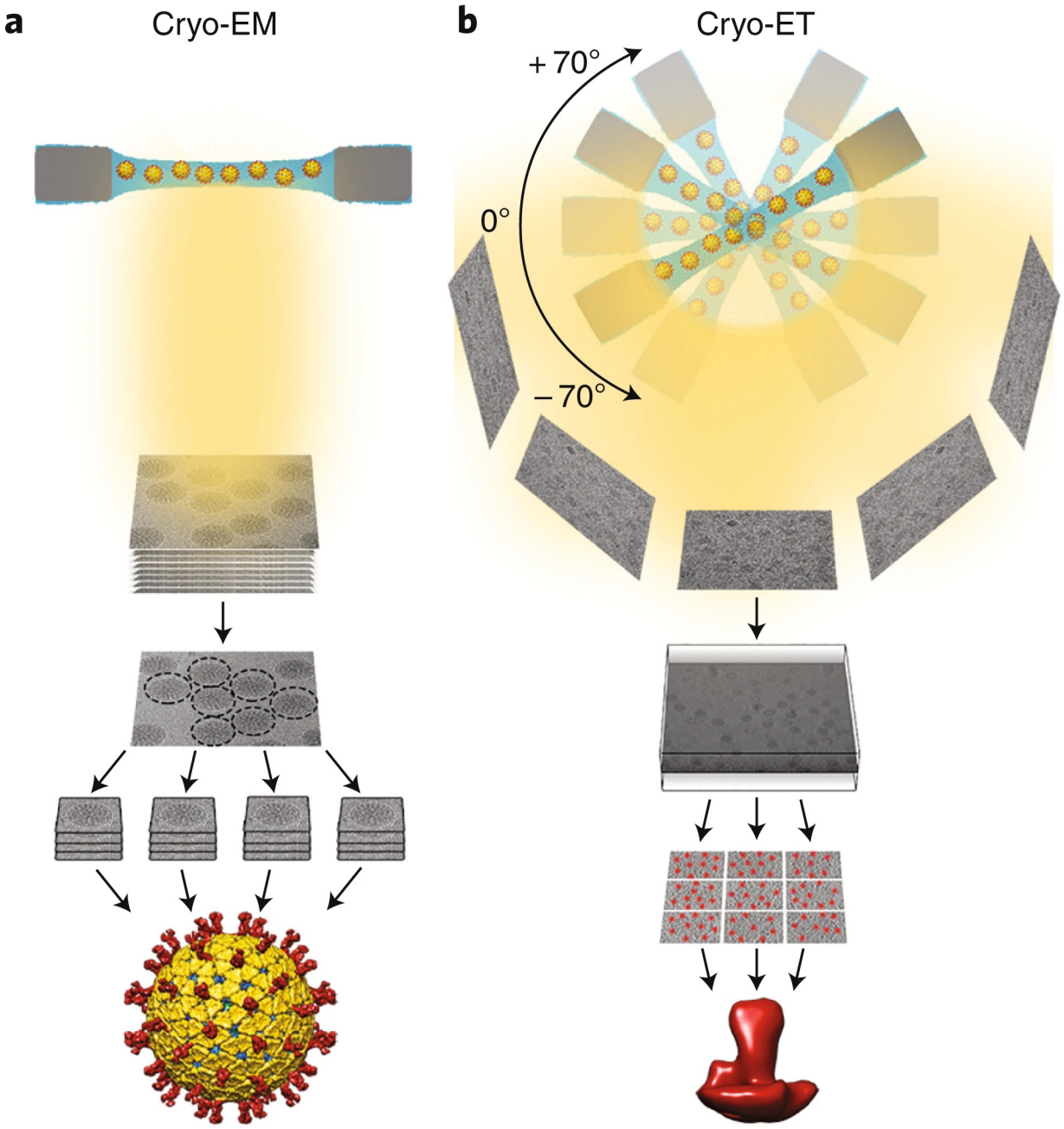
Electron Microscopy



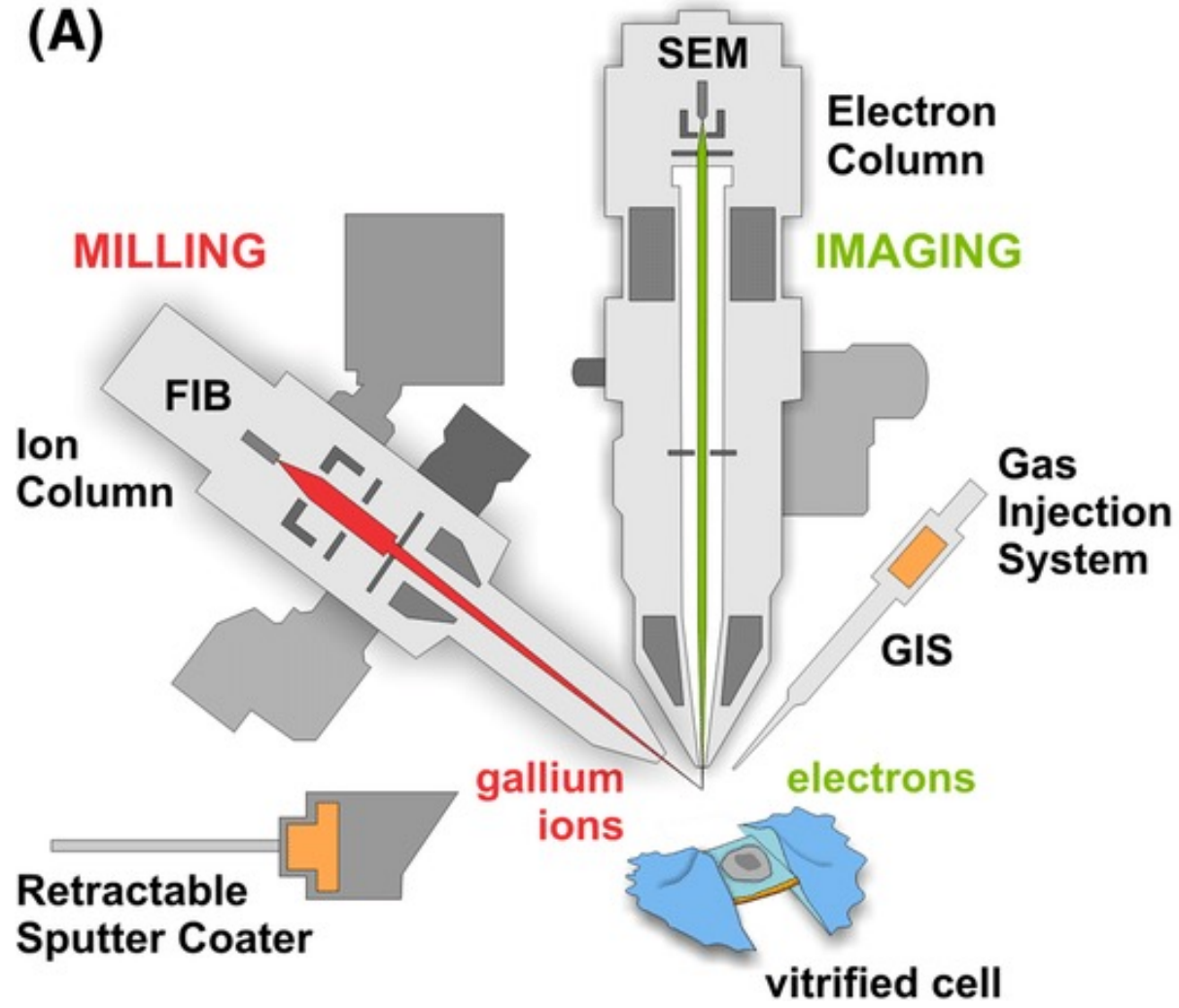


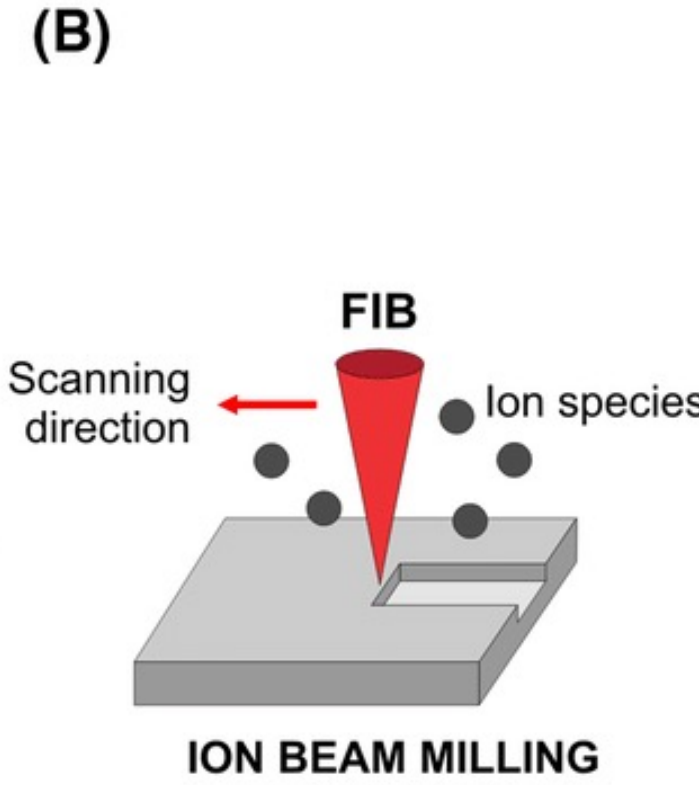


Electron Microscopy

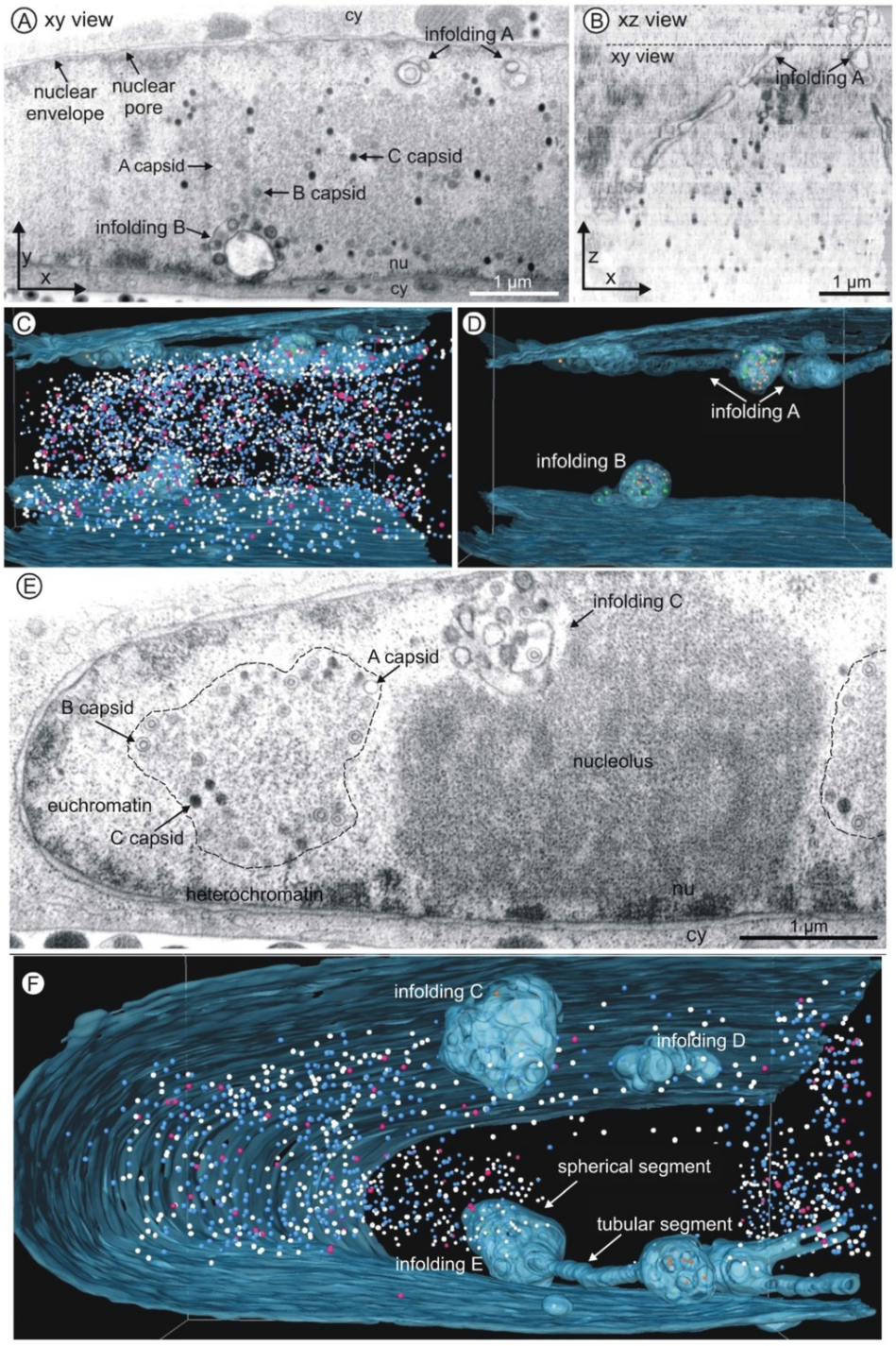


focused ion beam



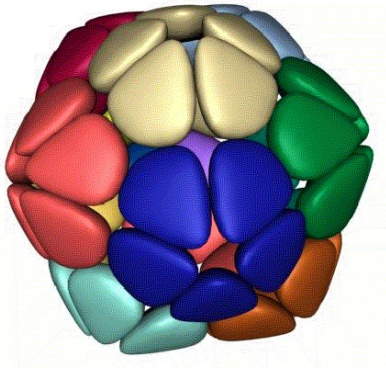


FIB/SEM tomography of an HCMV infected nucleus



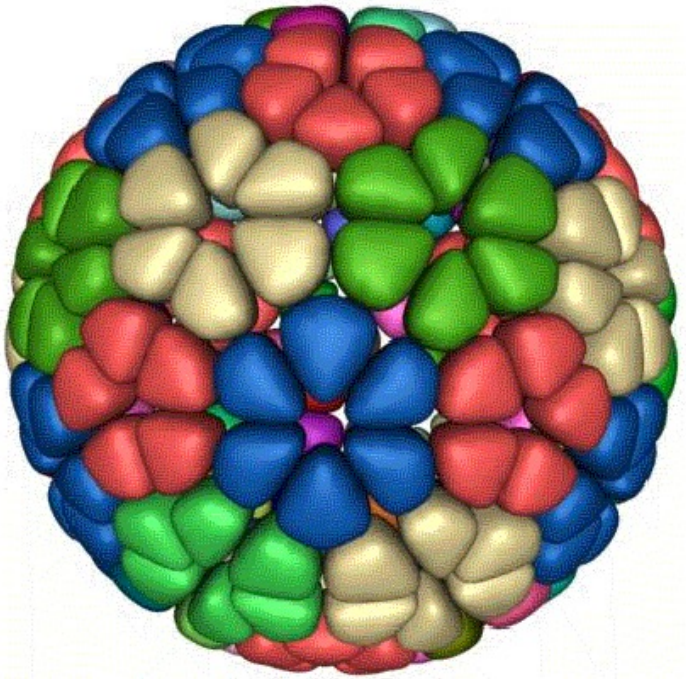
Quasi-equivalence

- Subunits are in “minimally” different environments
  - Pentamers at vertices
  - Hexamers elsewhere



- Predicts packing arrangements of larger capsids
  - Shift from T1 to T4 packing

  => 8-fold increase in volume



Goldberg diagram

$$T = h^2 + hk + k^2,$$

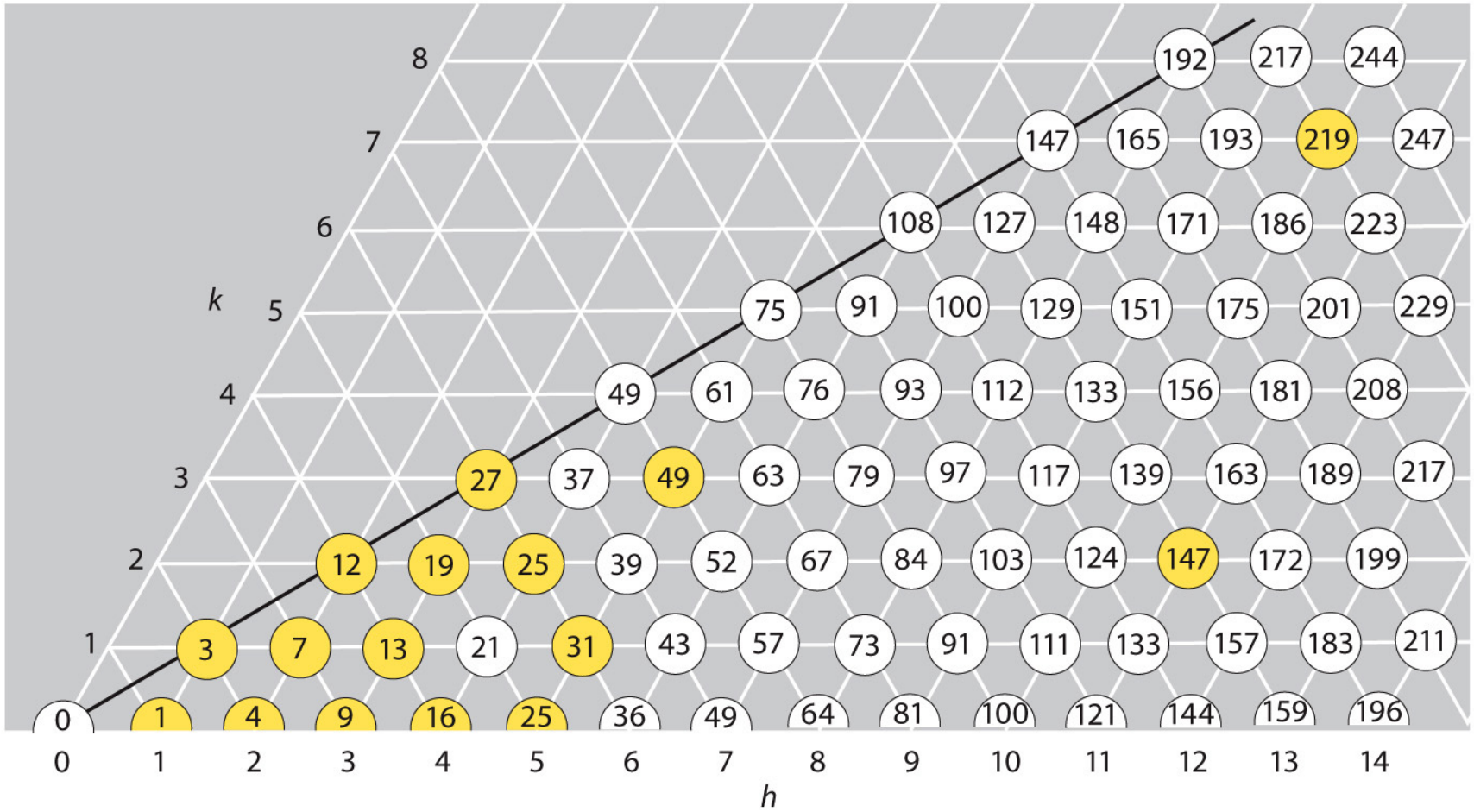
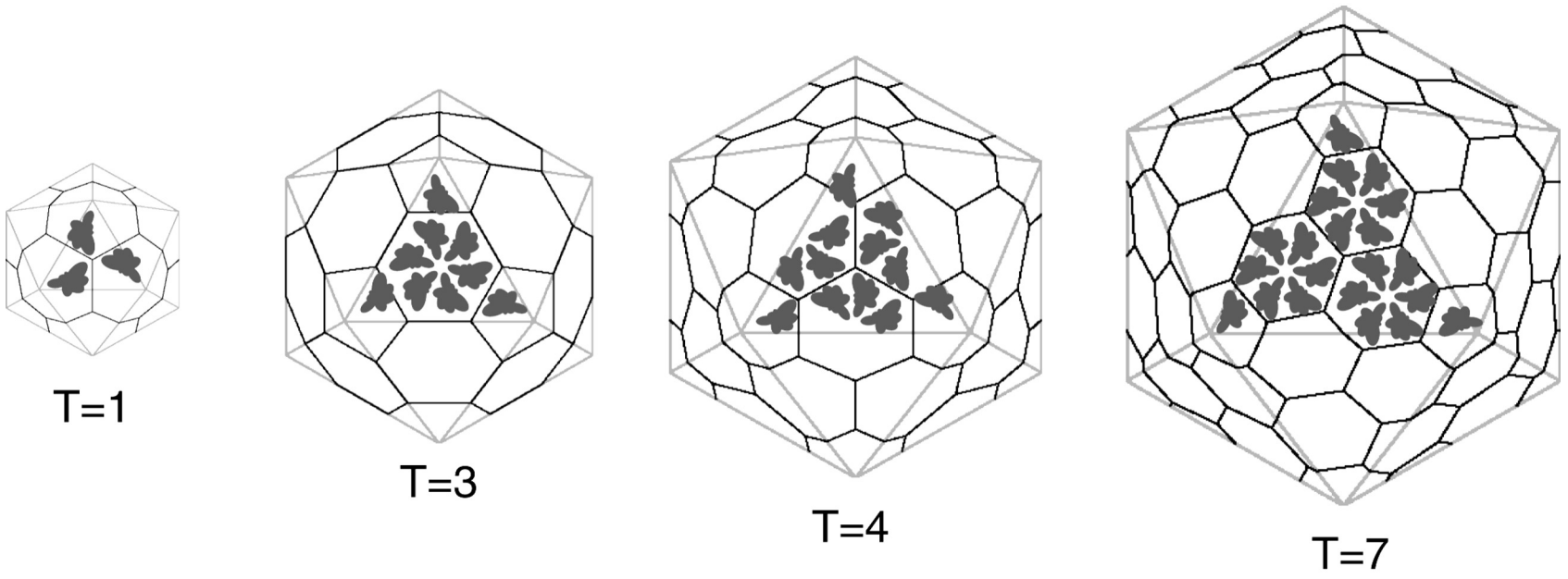


Figure 8.10 Molecular Biology of Assemblies and Machines (© Garland Science 2016)

Spherical viruses have icosahedral symmetry



Icosahedral capsids

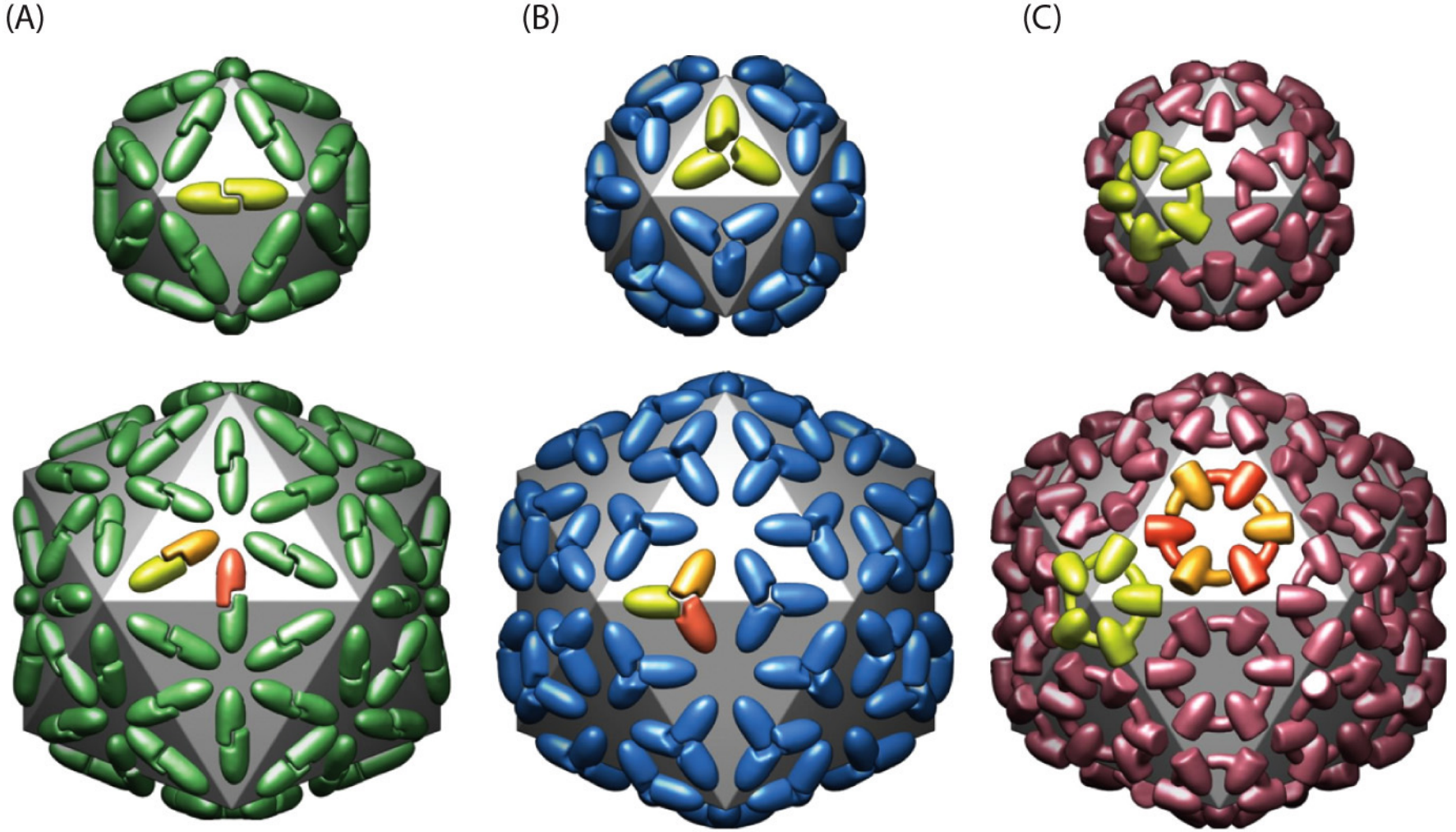


Figure 8.8 Molecular Biology of Assemblies and Machines (© Garland Science 2016)

interactions between complementary surface patches

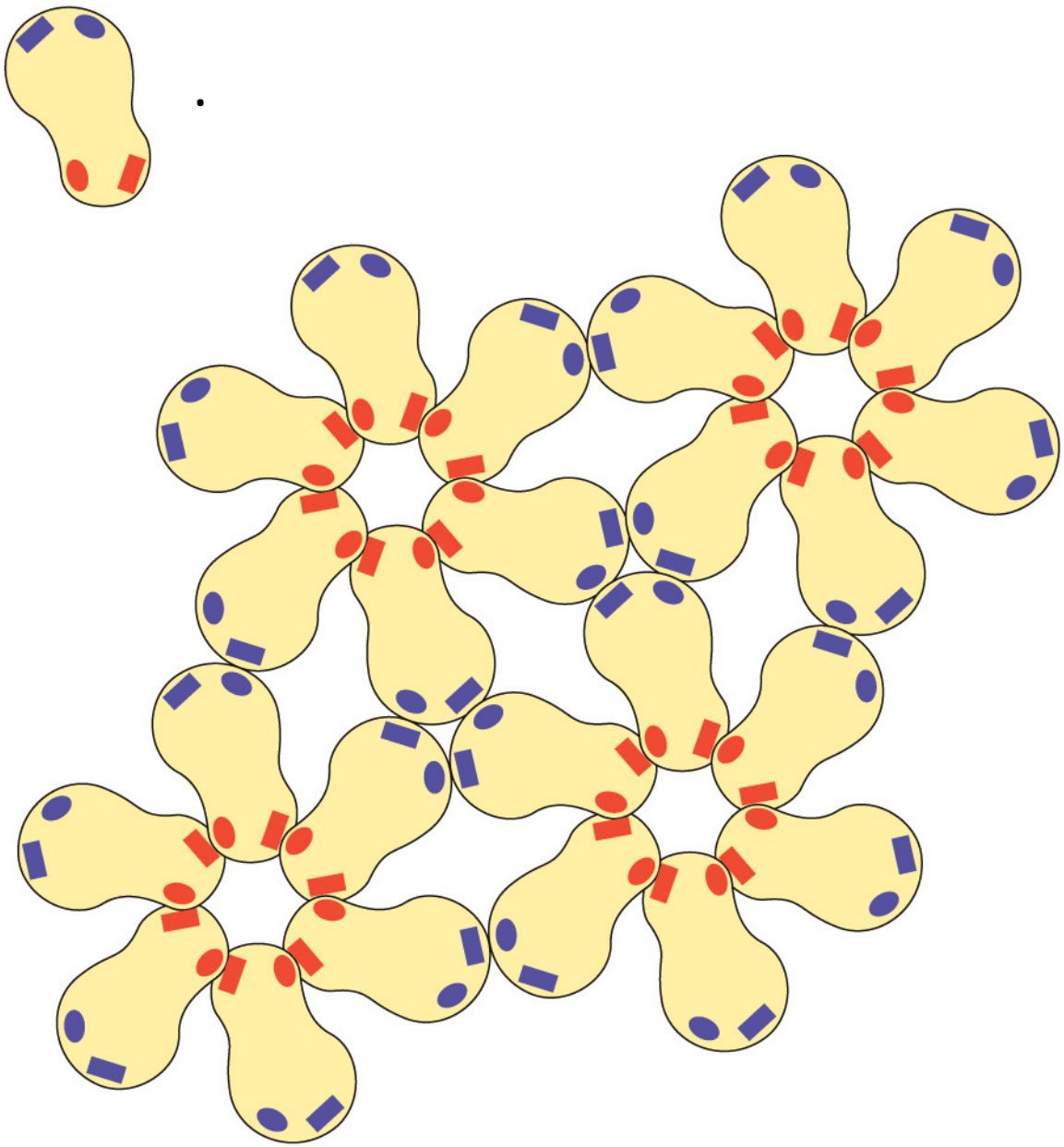


Figure 8.13 Molecular Biology of Assemblies and Machines (© Garland Science 2016)

Helical viruses

tobacco mosaic virus (TMV

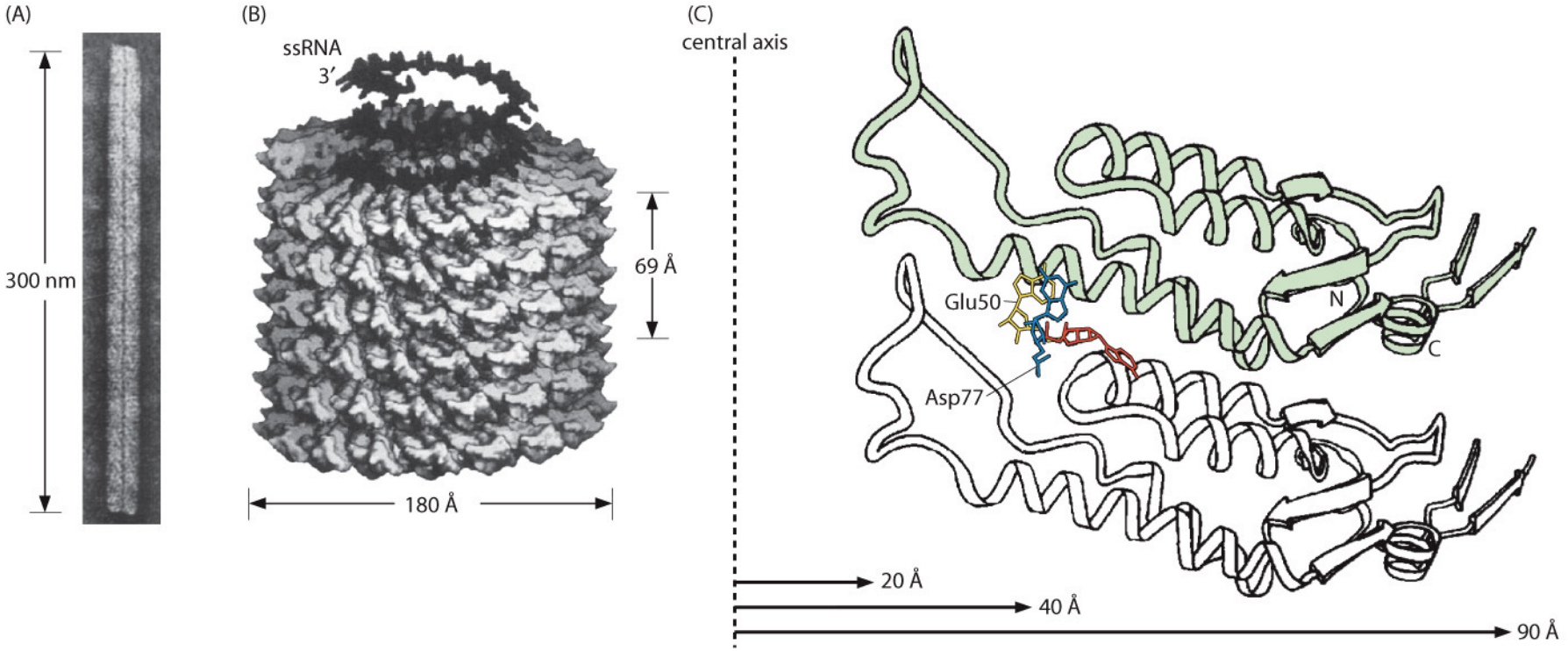


Figure 8.15 Molecular Biology of Assemblies and Machines (© Garland Science 2016)

Helical viruses

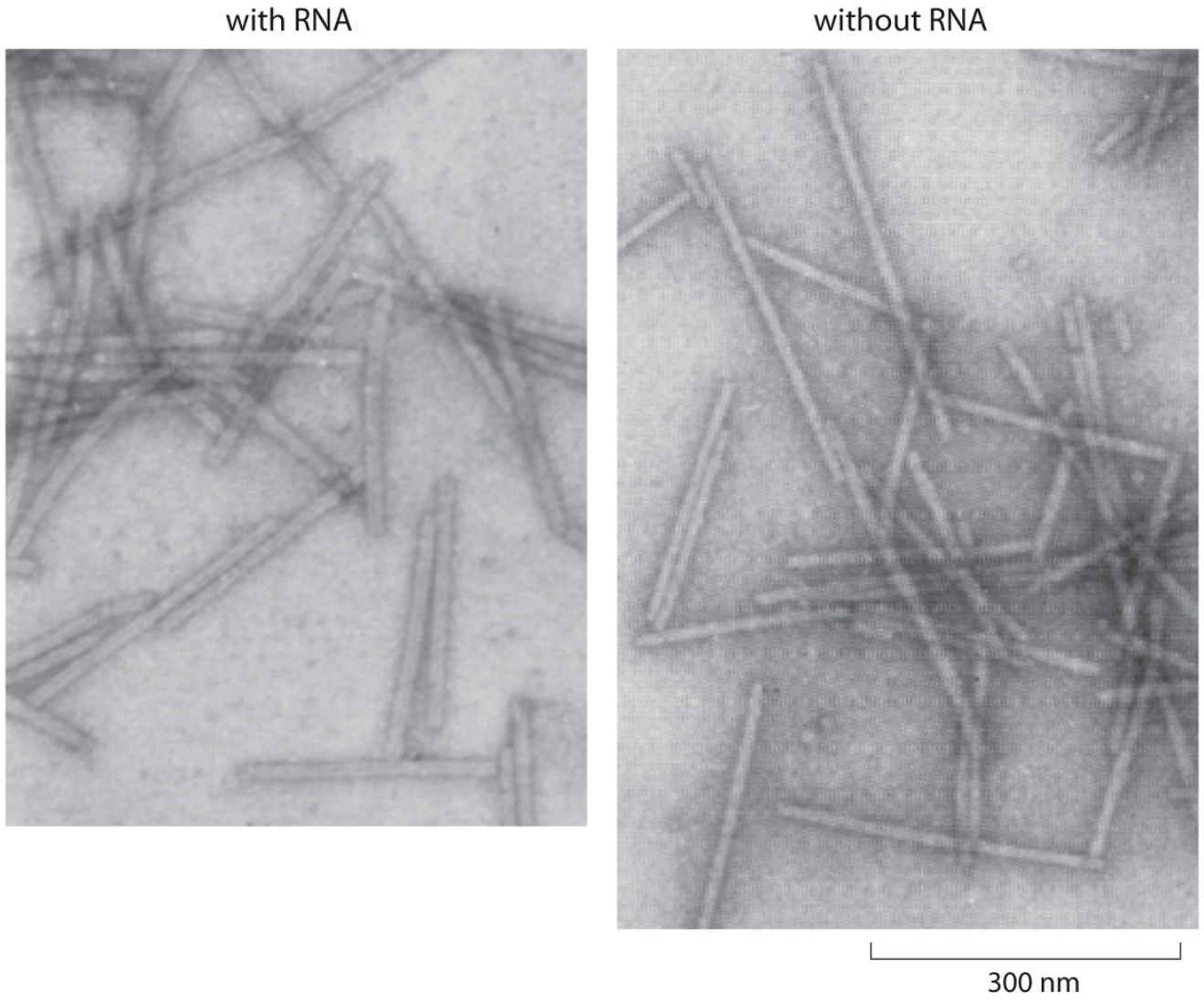


Figure 8.16 Molecular Biology of Assemblies and Machines (© Garland Science 2016)

Disassembly of TMV

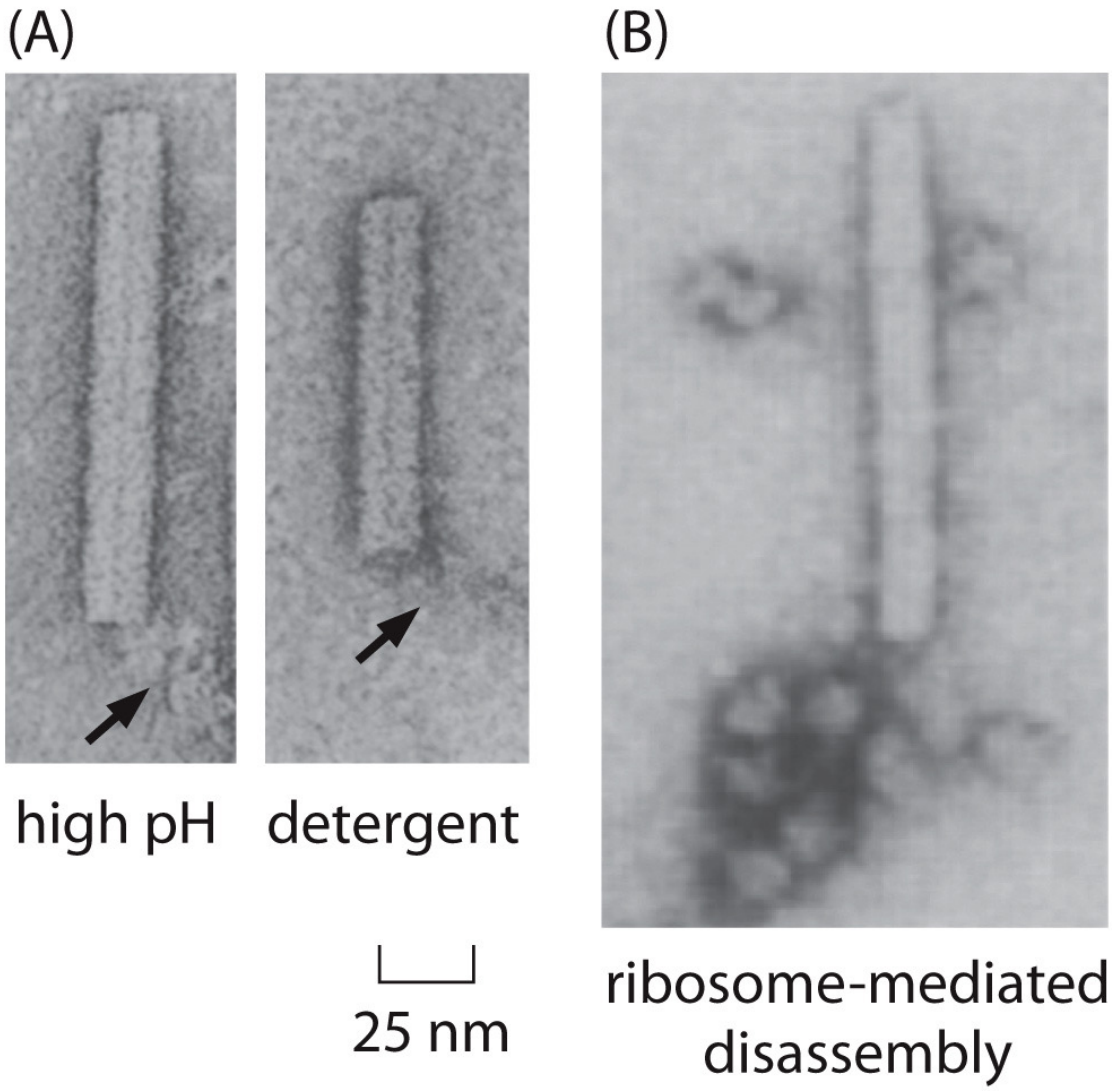


Figure 8.17 Molecular Biology of Assemblies and Machines (© Garland Science 2016)

Assembly of TMV

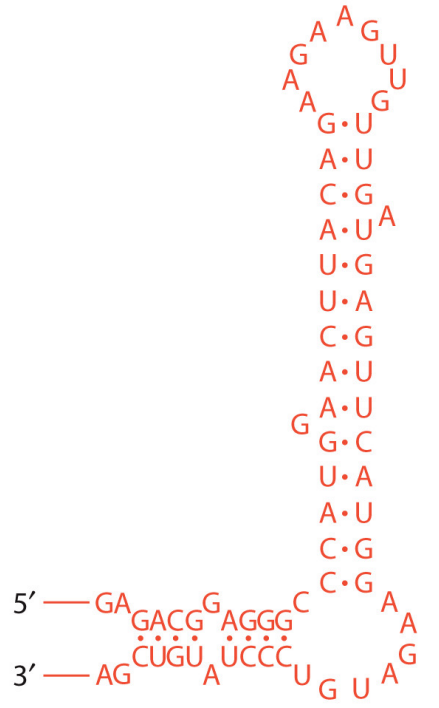
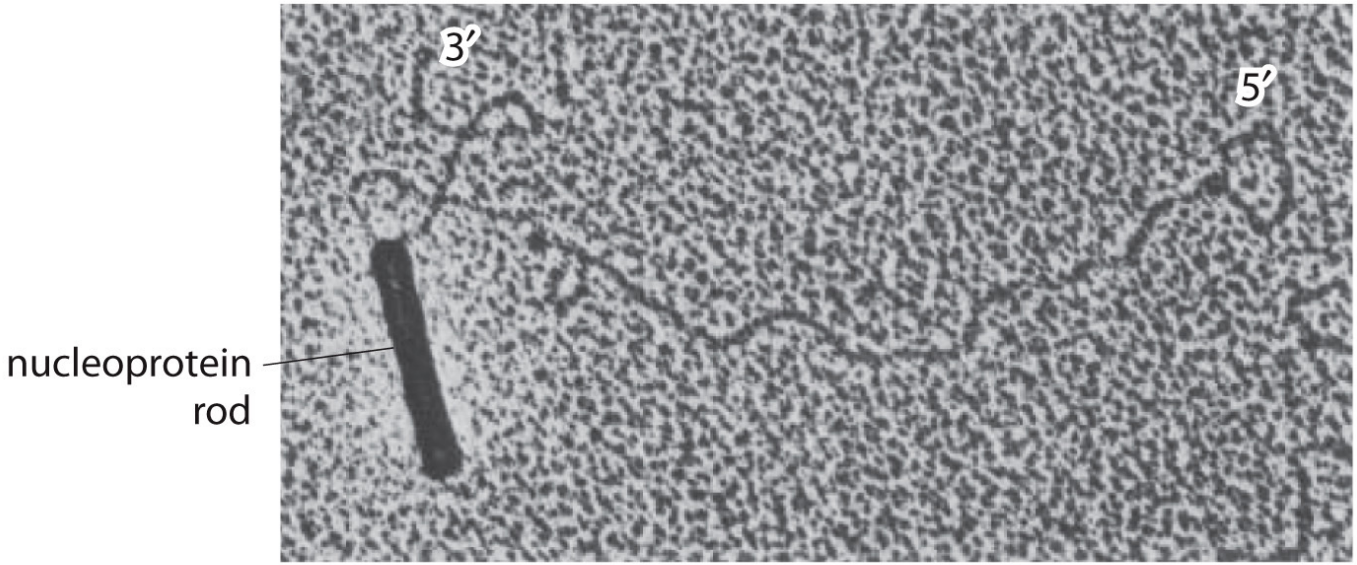


Figure 8.18a Molecular Biology of Assemblies and Machines (© Garland Science 2016)



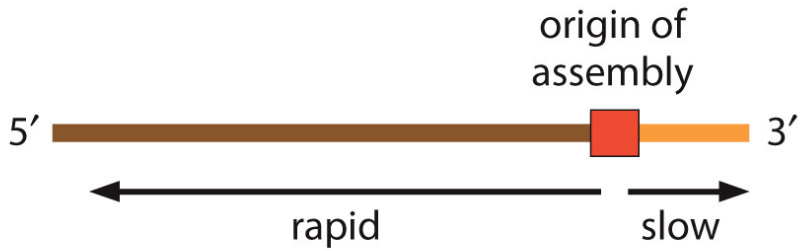


Figure 8.18b Molecular Biology of Assemblies and Machines (© Garland Science 2016)

Assembly of TMV

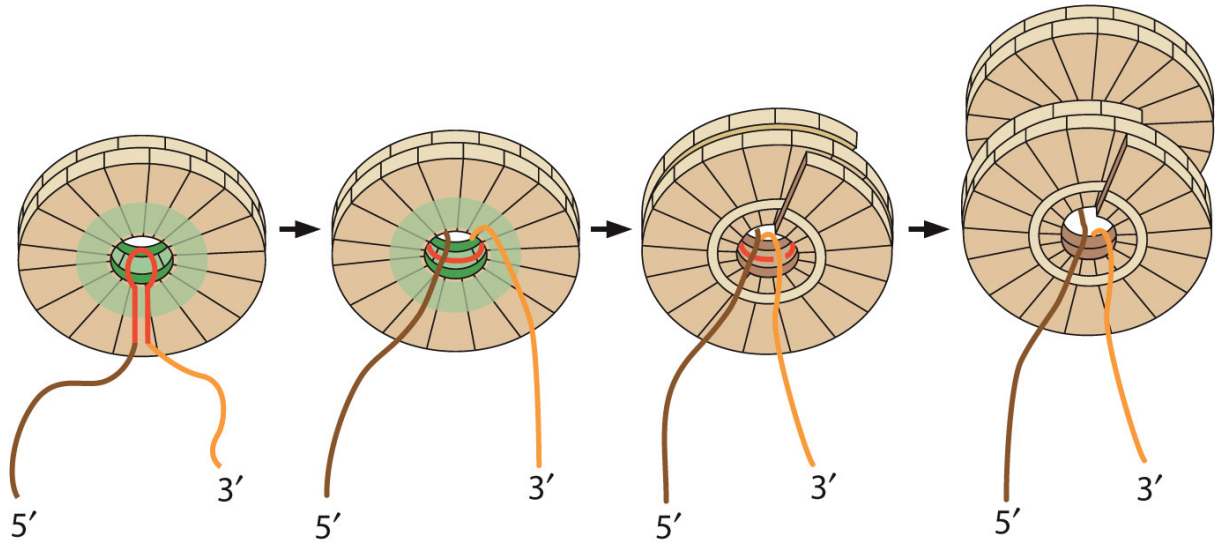


Figure 8.18c Molecular Biology of Assemblies and Machines (© Garland Science 2016)

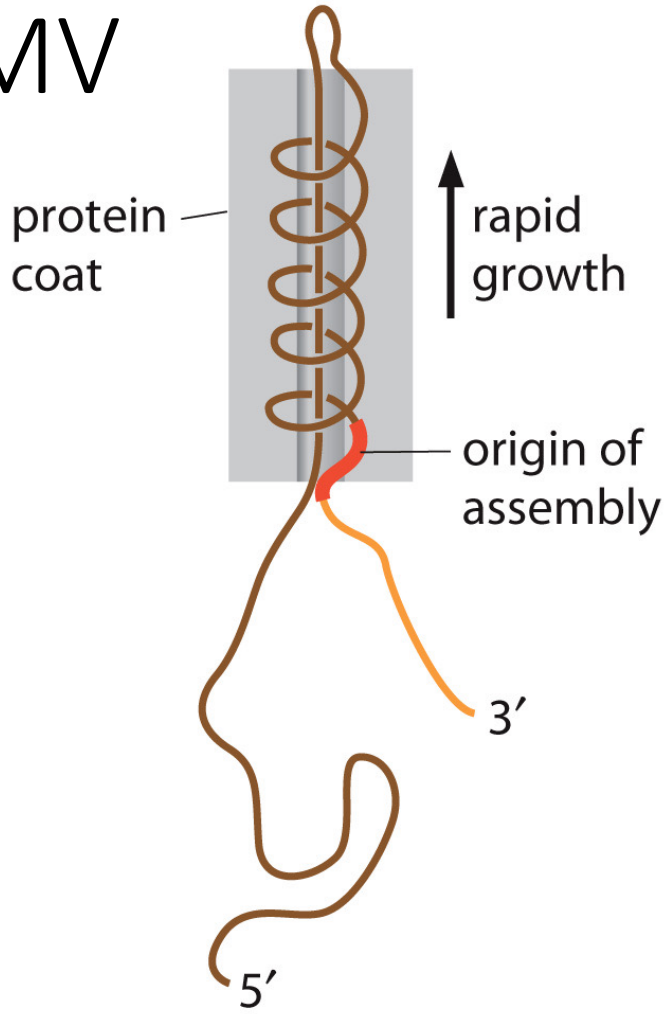
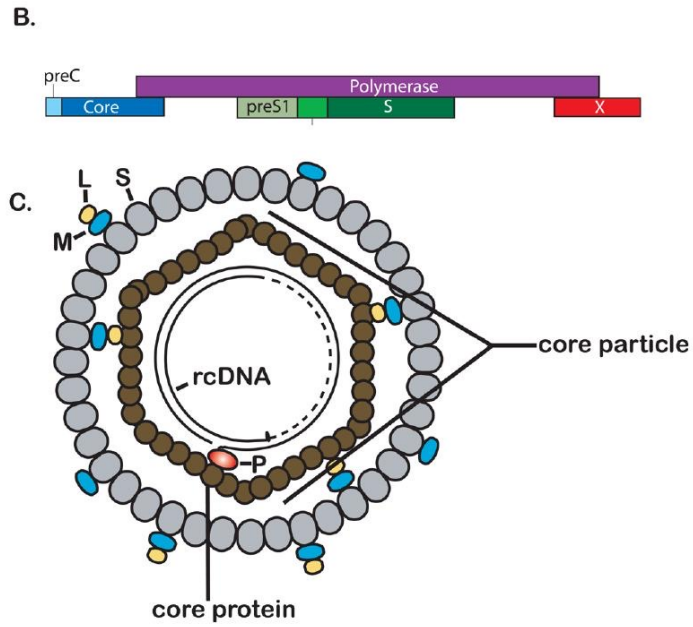
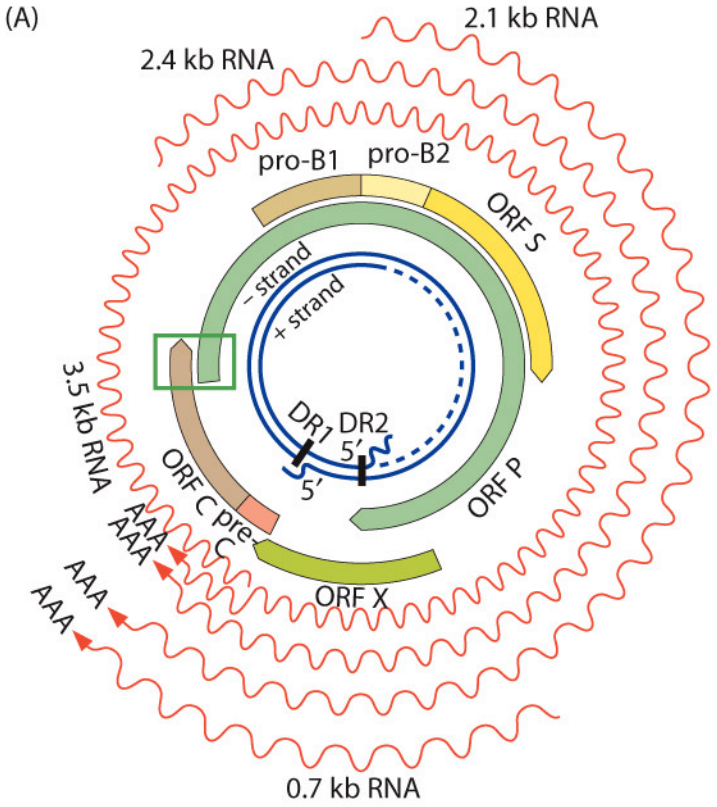


Figure 8.18d Molecular Biology of Assemblies and Machines (© Garland Science 2016)

small Icosahedral viruses

hepatitis B virus





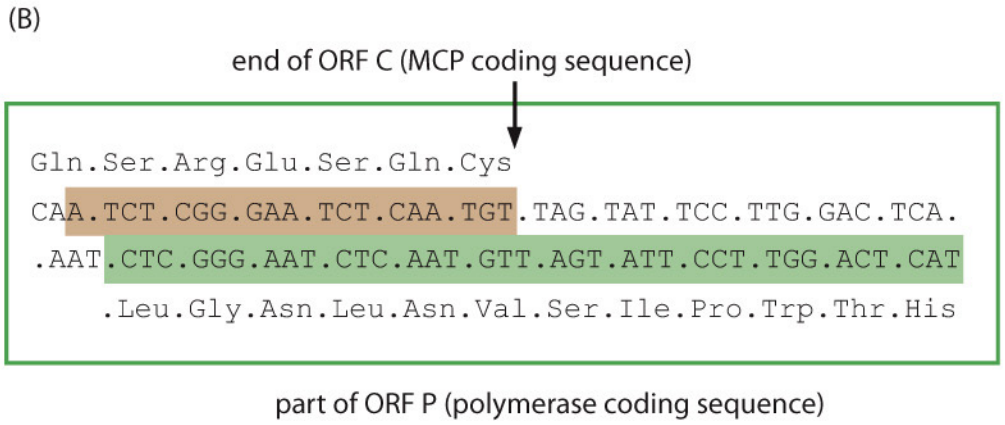


Figure 8.26 Molecular Biology of Assemblies and Machines (© Garland Science 2016)

Life cycle

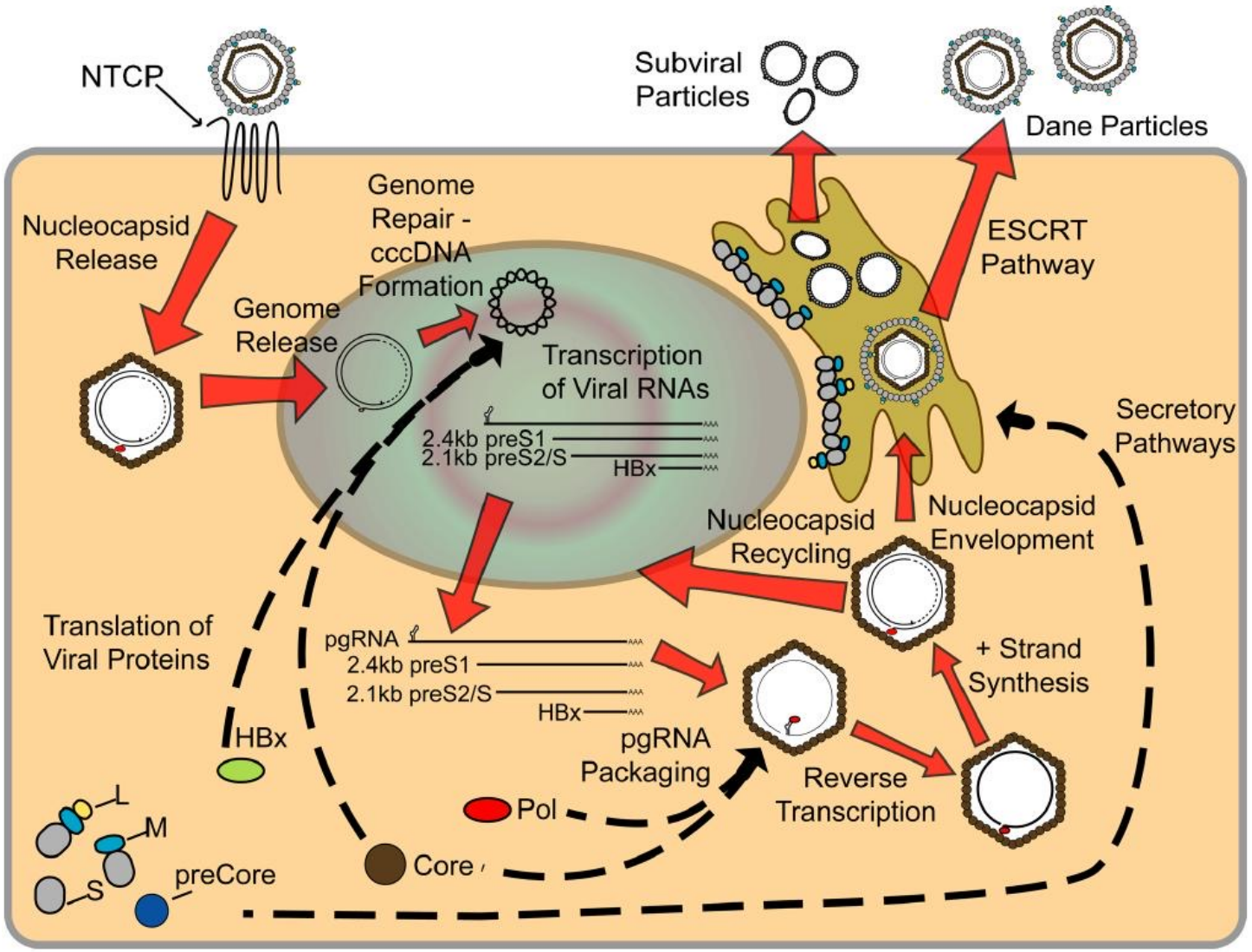
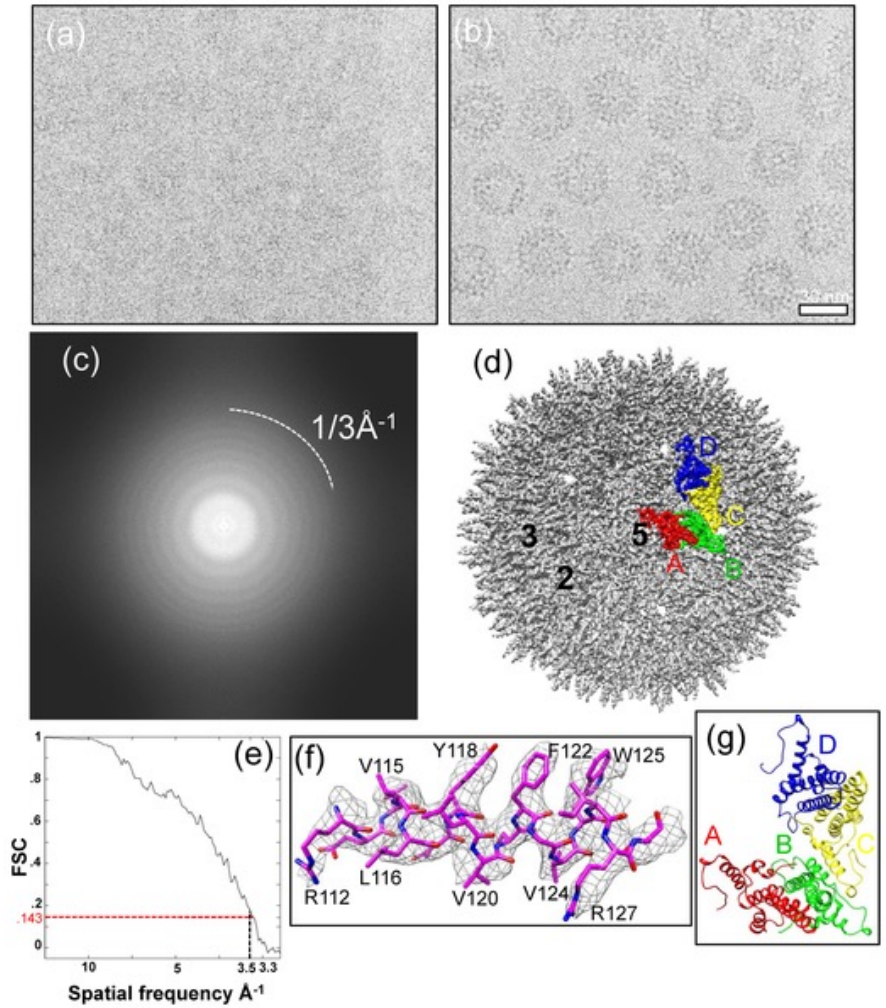
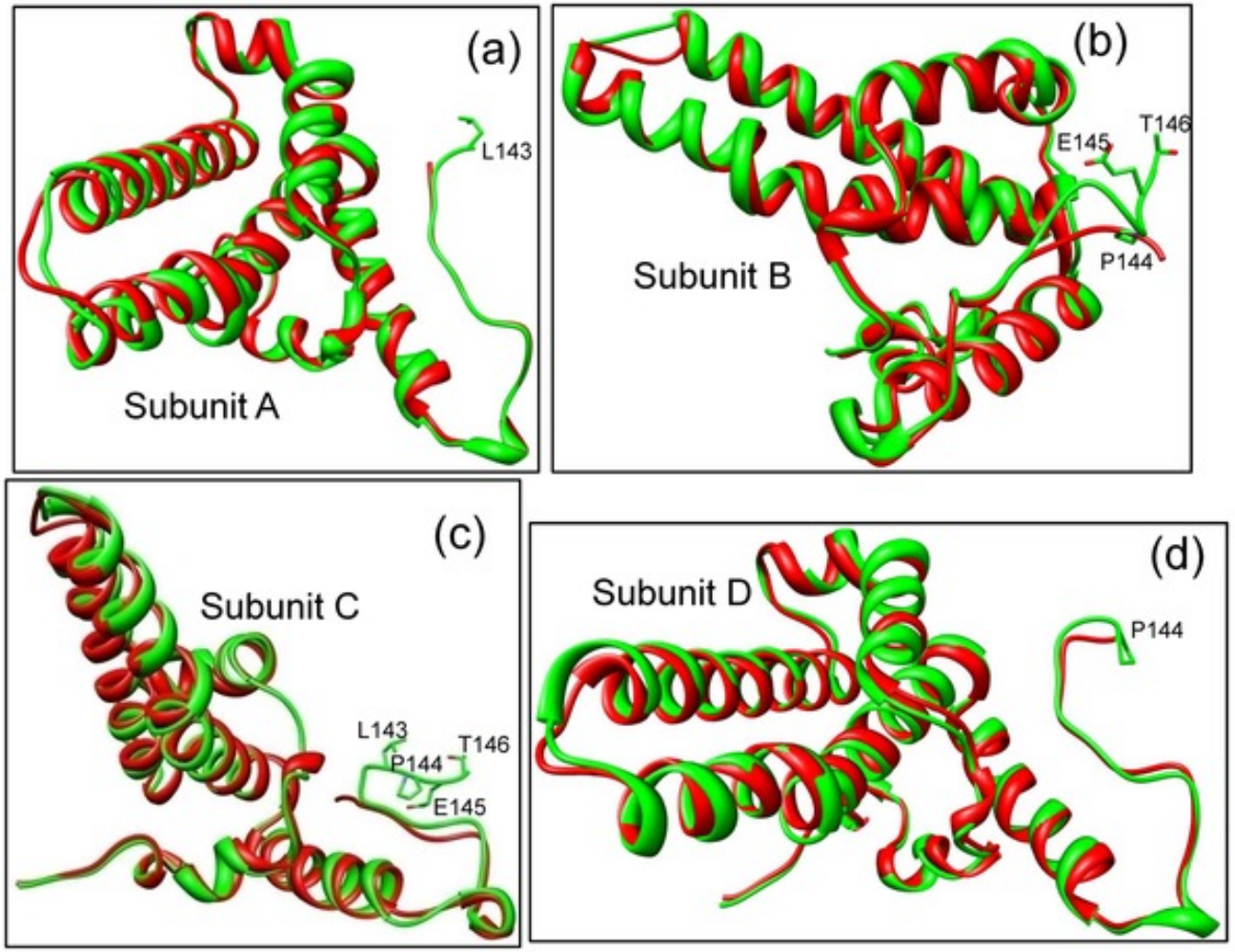

NTCP
Subviral Particles
Dane Particles
Nucleocapsid Release
Genome Repair - cccDNA Formation
Genome Release
Transcription of Viral RNAs
ESCRT Pathway
2.4kb preS1
2.1kb preS2/S
HBx
Secretory Pathways
Nucleocapsid Recycling
Nucleocapsid Envelopment
Translation of Viral Proteins
pgRNA
2.4kb preS1
2.1kb preS2/S
HBx
+ Strand Synthesis
HBx
pgRNA Packaging
Reverse Transcription
Pol
L
M
S
preCore
Core

Figure 1. CryoEM and 3D reconstruction of hepatitis B virus (HBV) core assembled from full-length HBV core proteins at 3.5Å resolution.



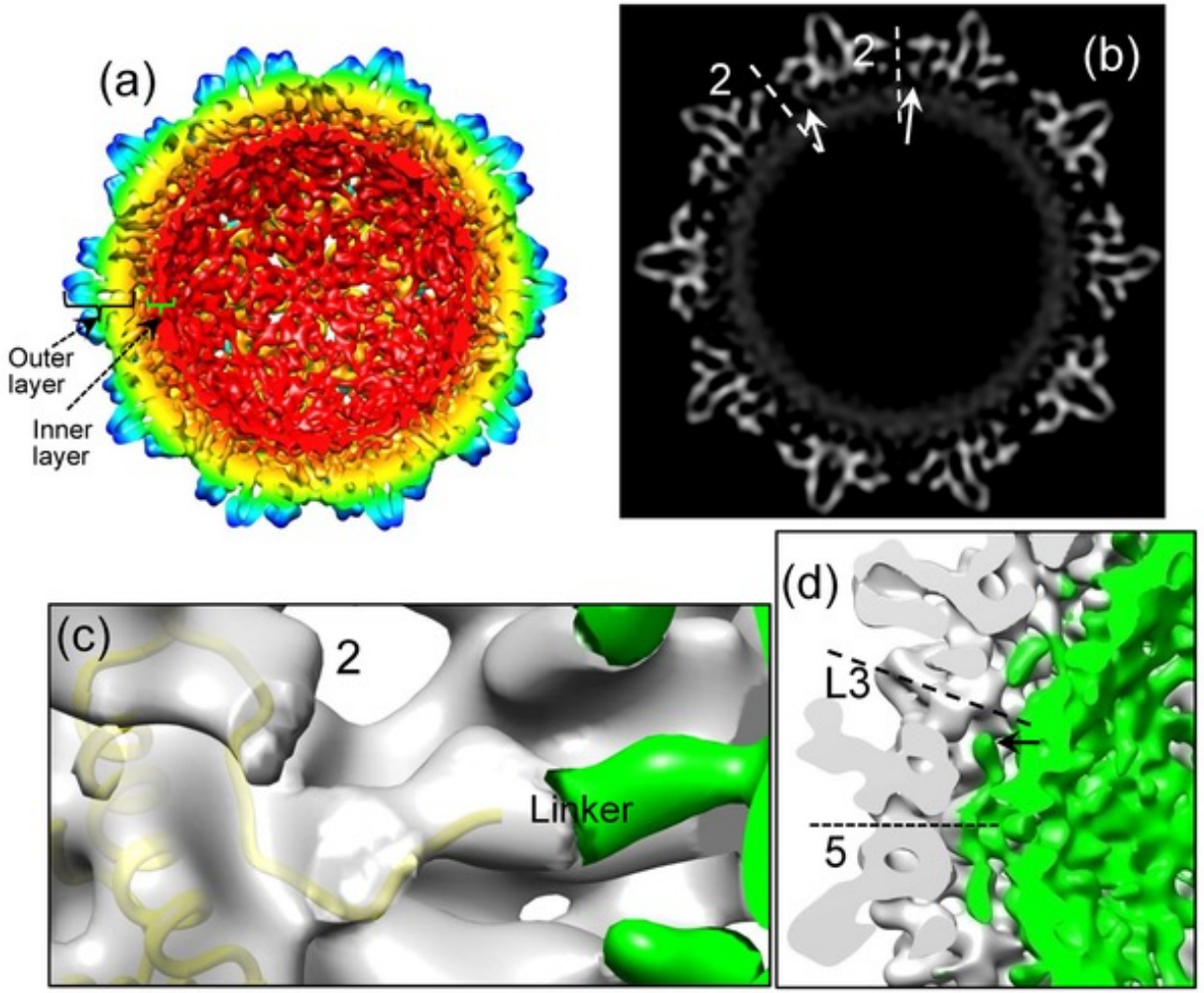
Yu X, Jin L, Jih J, Shih C, Hong Zhou Z (2013) 3.5Å cryoEM Structure of Hepatitis B Virus Core Assembled from Full-Length Core Protein. PLOS ONE 8(9): e69729. https://doi.org/10.1371/journal.pone.0069729
https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0069729

Figure 2. Comparisons between corresponding cryoEM structures (green) and crystal structures (red) by superimposition.



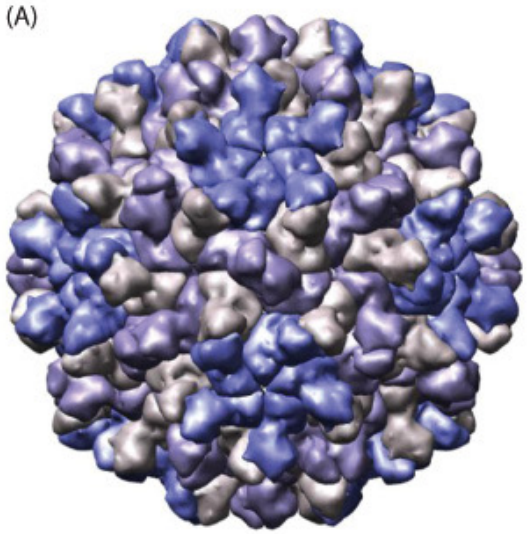
Yu X, Jin L, Jih J, Shih C, Hong Zhou Z (2013) 3.5Å cryoEM Structure of Hepatitis B Virus Core Assembled from Full-Length Core Protein. PLOS ONE 8(9): e69729. https://doi.org/10.1371/journal.pone.0069729
https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0069729

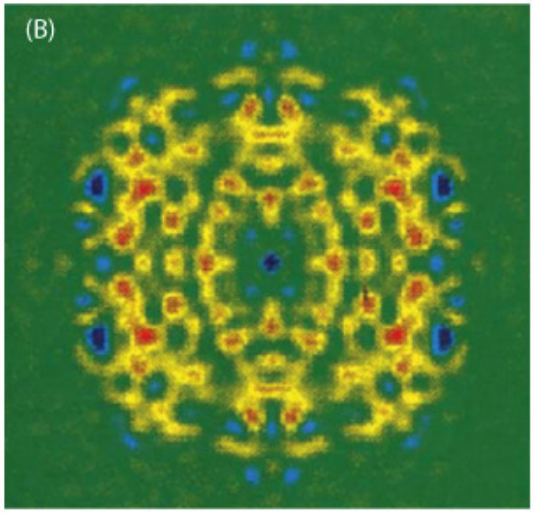
Figure 4. Maps of HBV core reconstruction filtered to 10Å resolution.



Yu X, Jin L, Jih J, Shih C, Hong Zhou Z (2013) 3.5Å cryoEM Structure of Hepatitis B Virus Core Assembled from Full-Length Core Protein. PLOS ONE 8(9): e69729. https://doi.org/10.1371/journal.pone.0069729
https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0069729

neutron diffraction





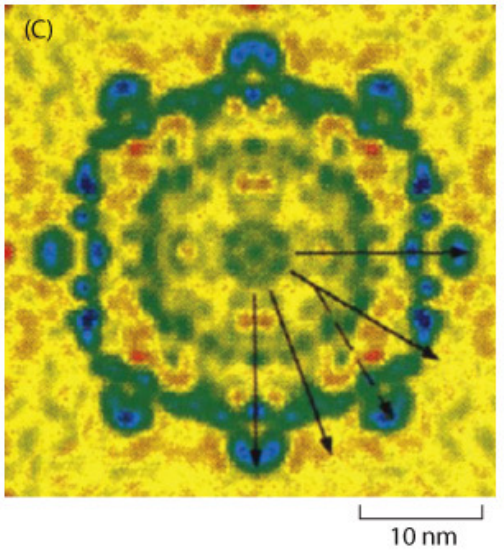
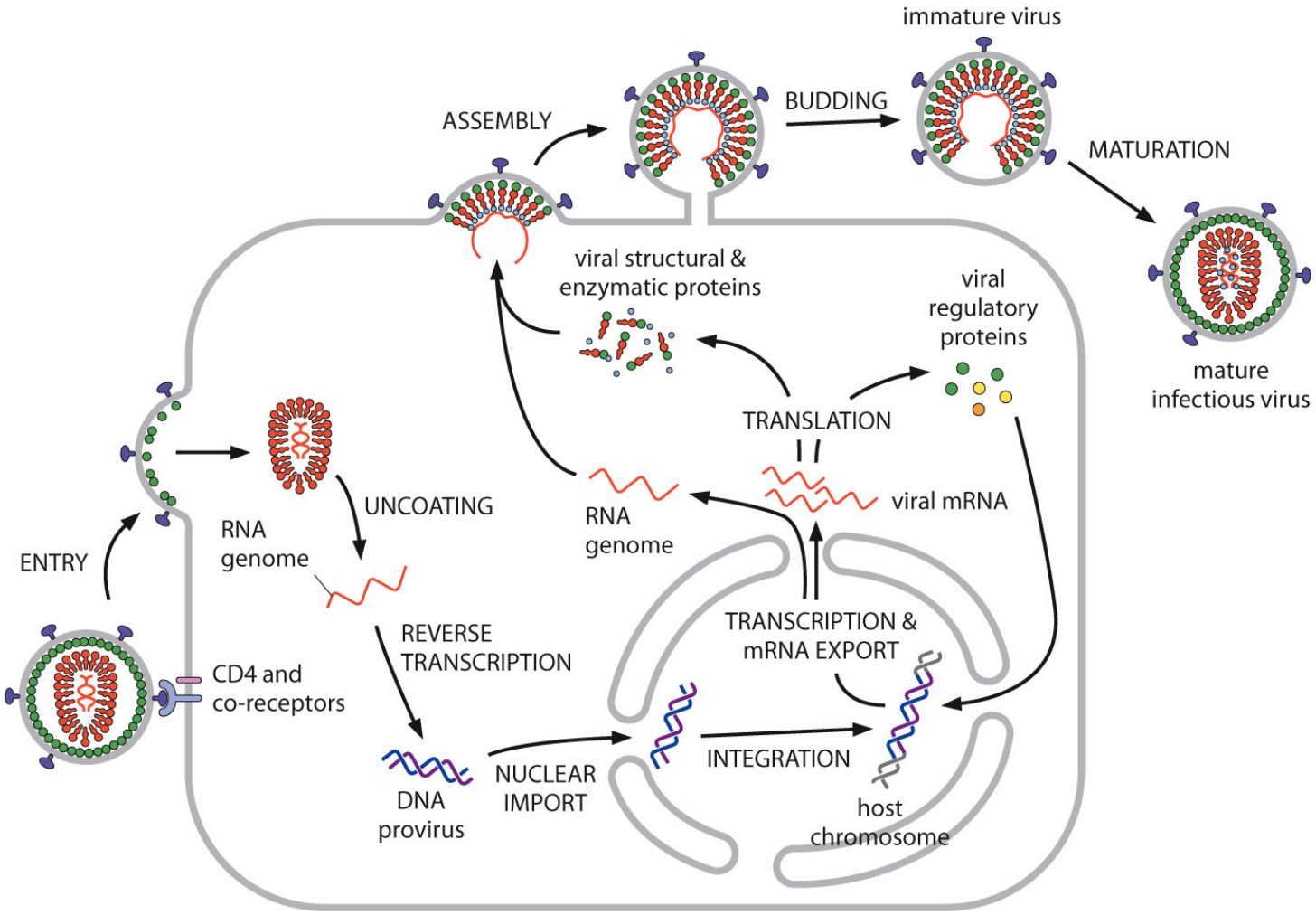


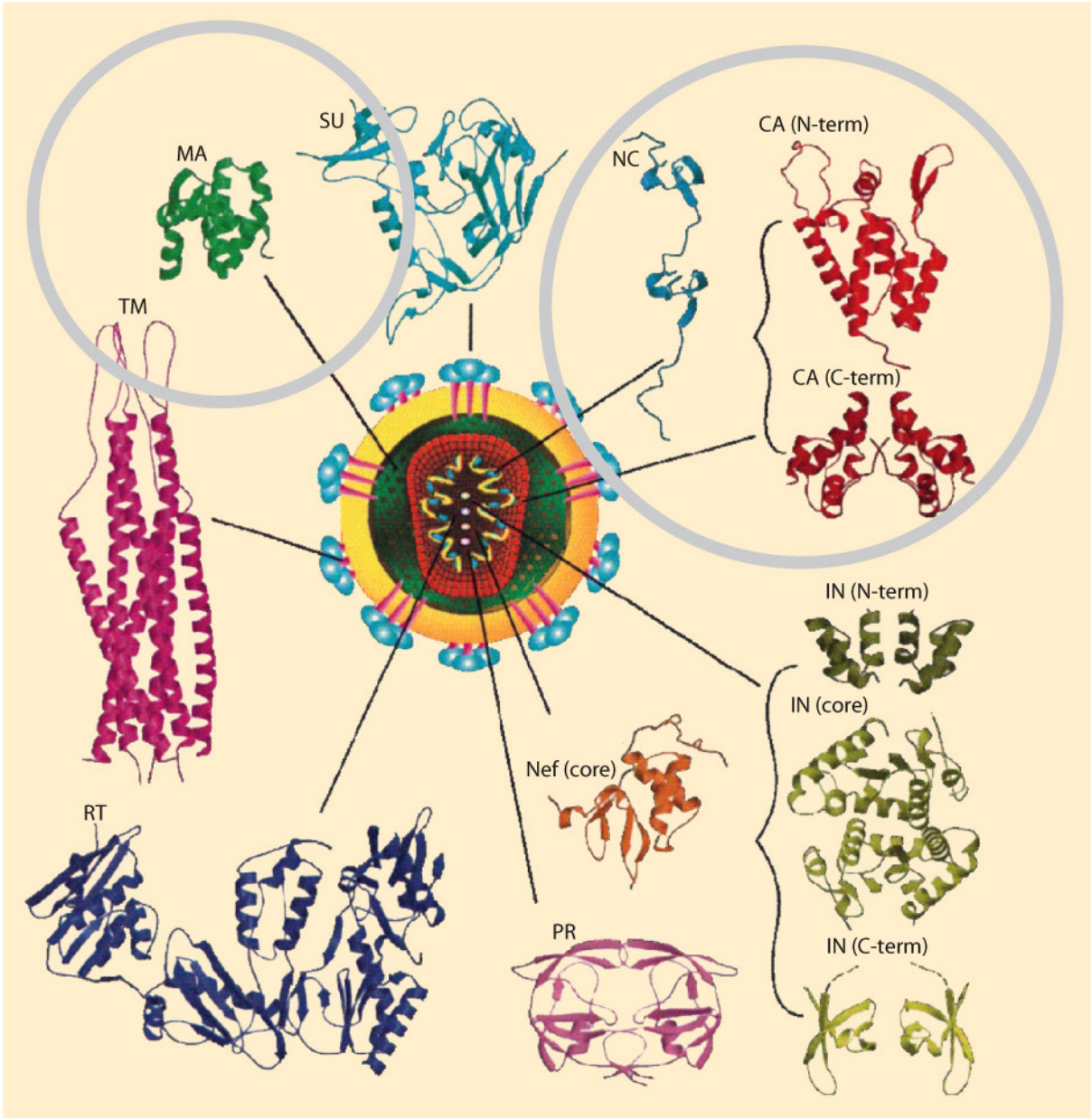
Figure 8.28 Molecular Biology of Assemblies and

tomato bushy stunt virus (TBSV)

assembly and maturation of human immunodeficiency virus (HIV)



Box 8.1 Figure 8.1.1 Molecular Biology of Assemblies and Machines (© Garland Science 2016)



Box 8.1 Figure 8.1.2 Molecular Biology of Assemblies and Machines (© Garland Science 2016)

Influenza virus

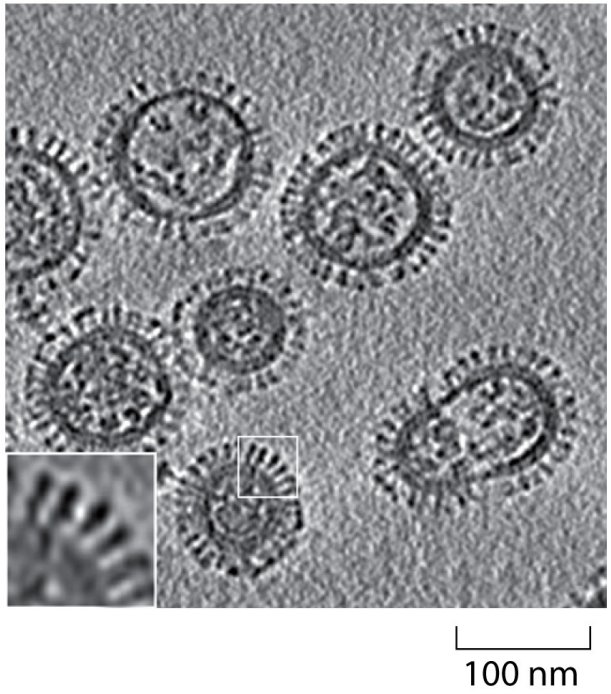


Figure 8.60a Molecular Biology of Assemblies and Machines (© Garland Science 2016)

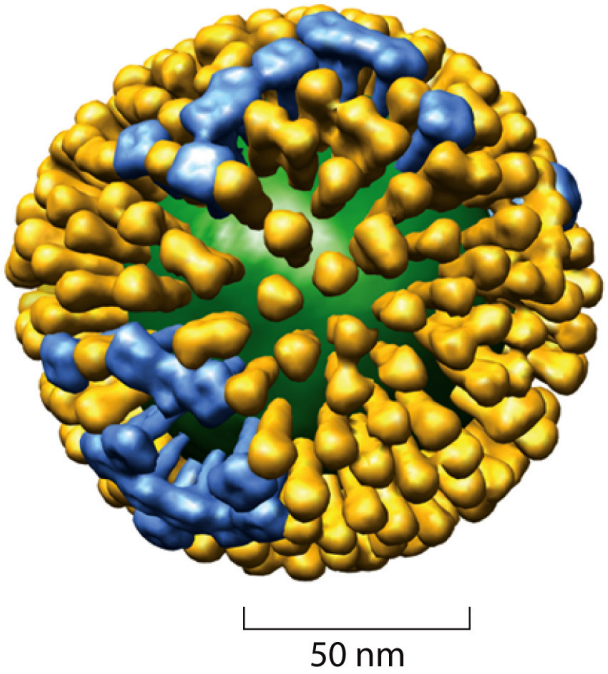


Figure 8.60b Molecular Biology of Assemblies and Machines (© Garland Science 2016)

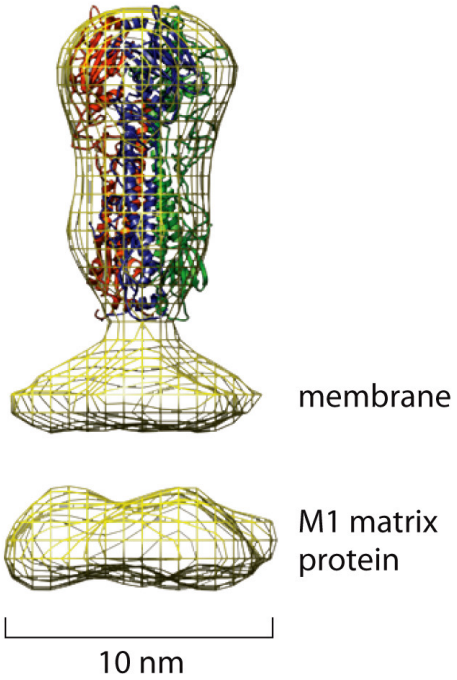
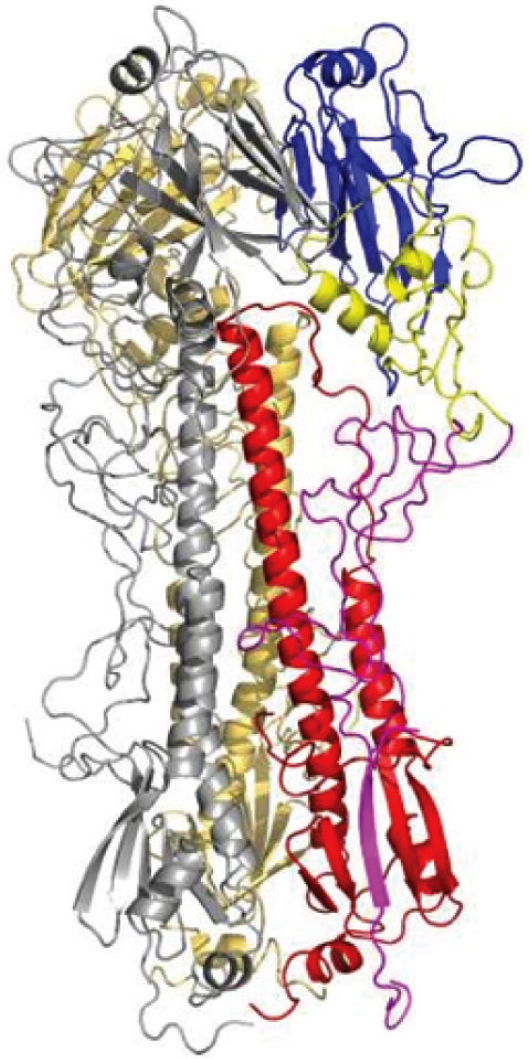
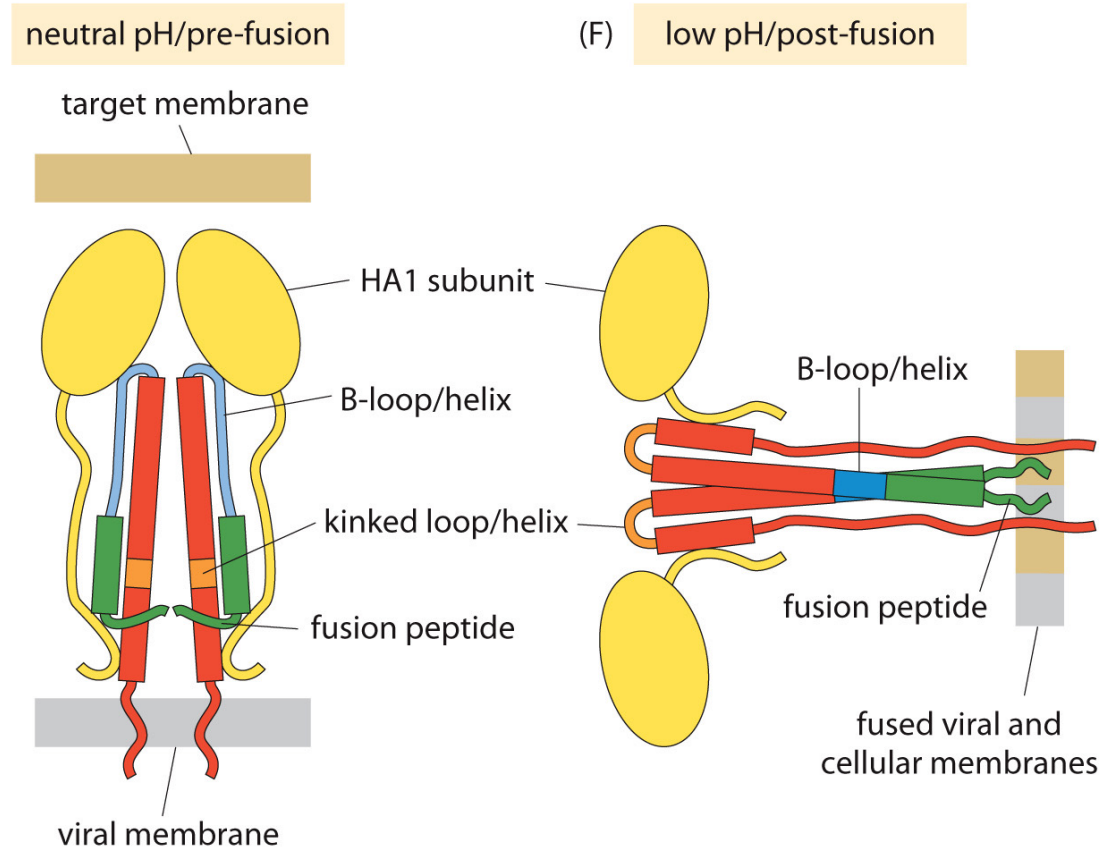


Figure 8.60c Molecular Biology of Assemblies and Machines (© Garland Science 2016)





.60ef Molecular Biology of Assemblies and Machines (© Garland Science 2016)

Figure 8.60d Molecular Biology of Assemblies and Machines (© Garland Science 2016)

Assembly pathway of influenza virus.

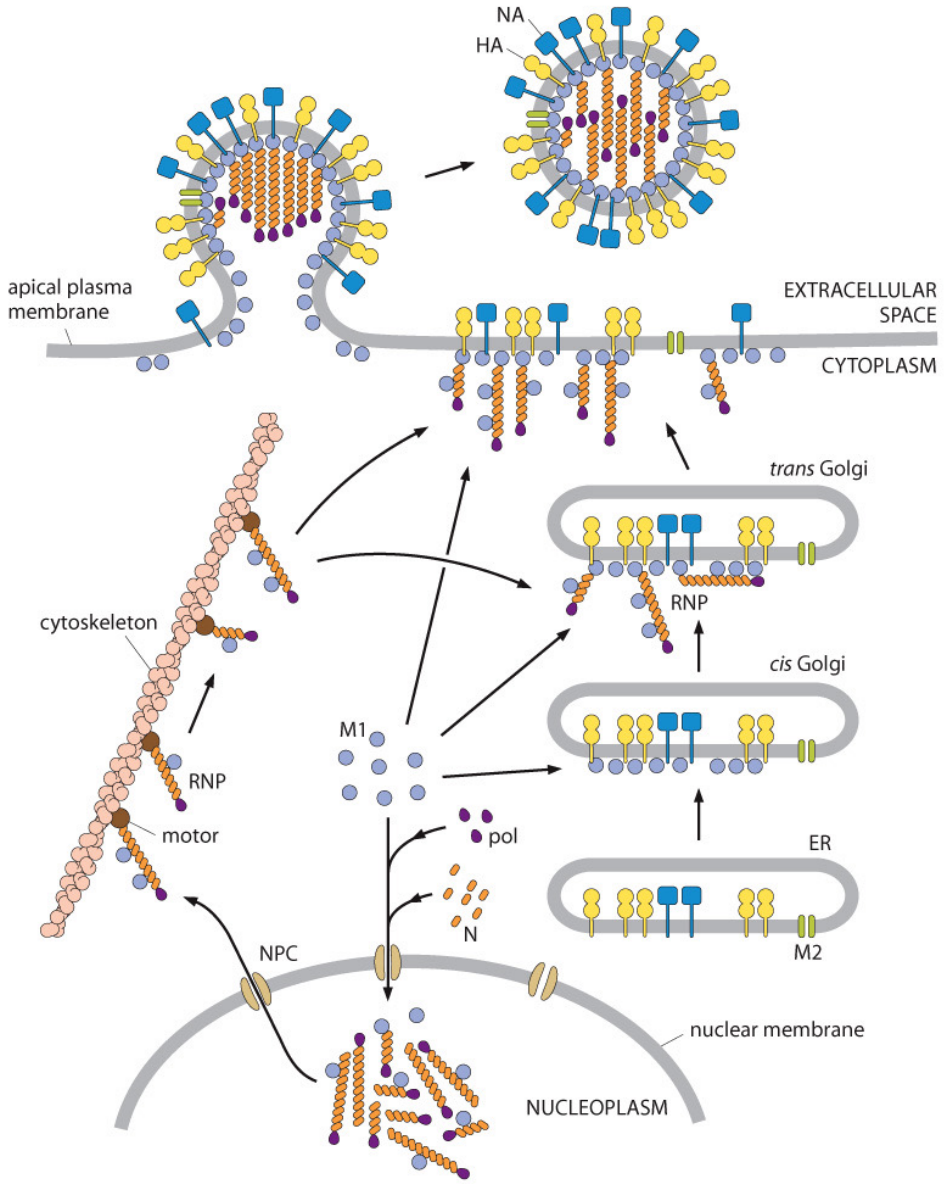
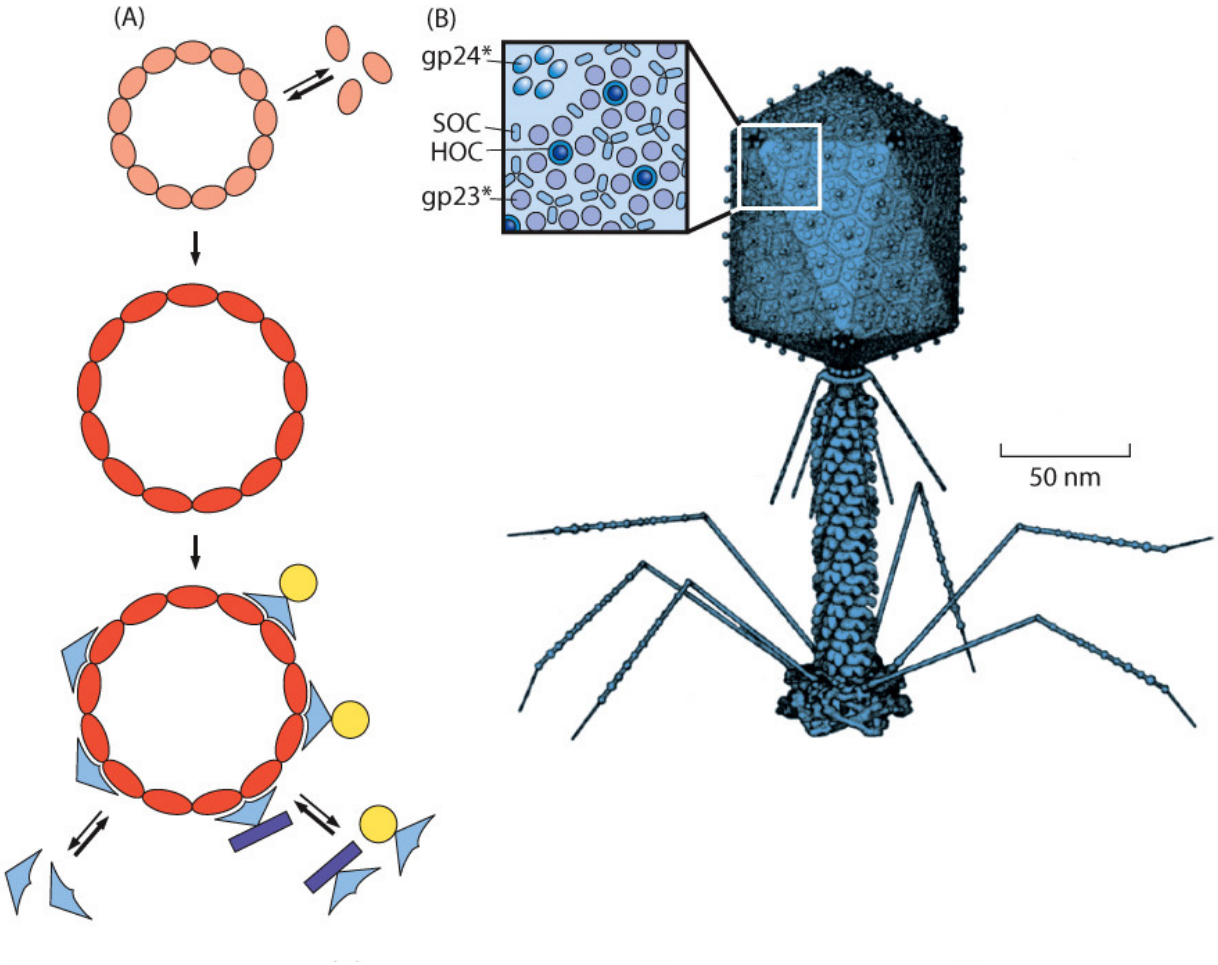


Figure 8.62 Molecular Biology of Assemblies and Machines (© Garland Science 2016)

Display of proteins on accessory proteins of dsDNA bacteriophages



Display of proteins on accessory proteins of dsDNA bacteriophages

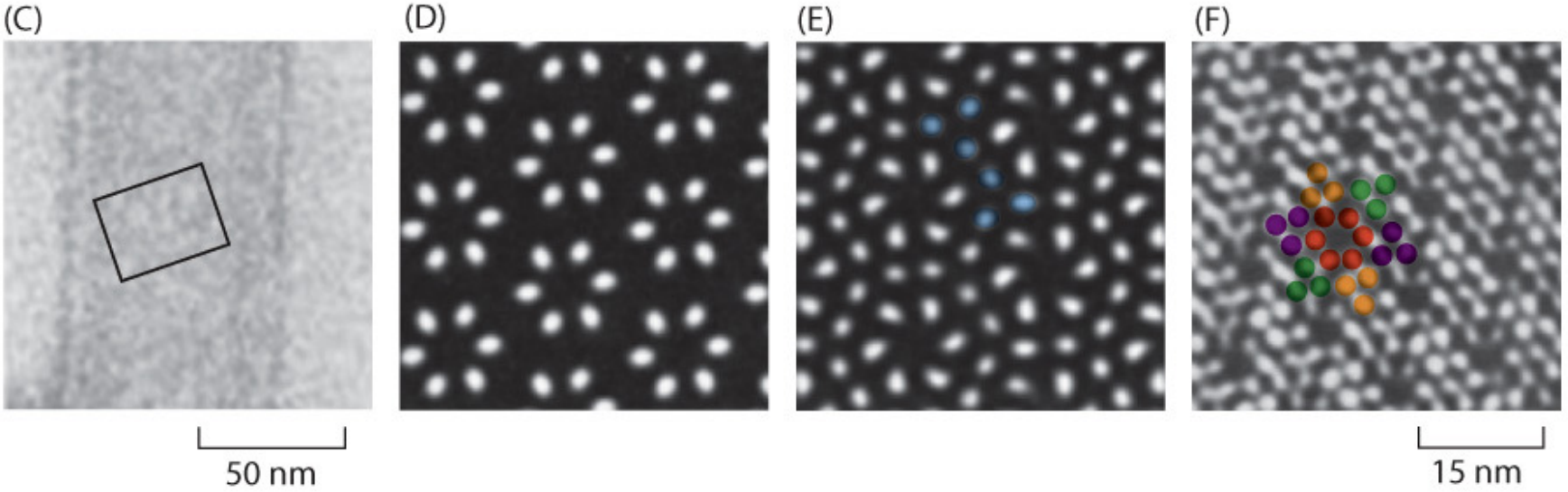


Figure 8.67 Molecular Biology of Assemblies and Machines (© Garland Science 2016)

Display of an Ig domain

flock house virus

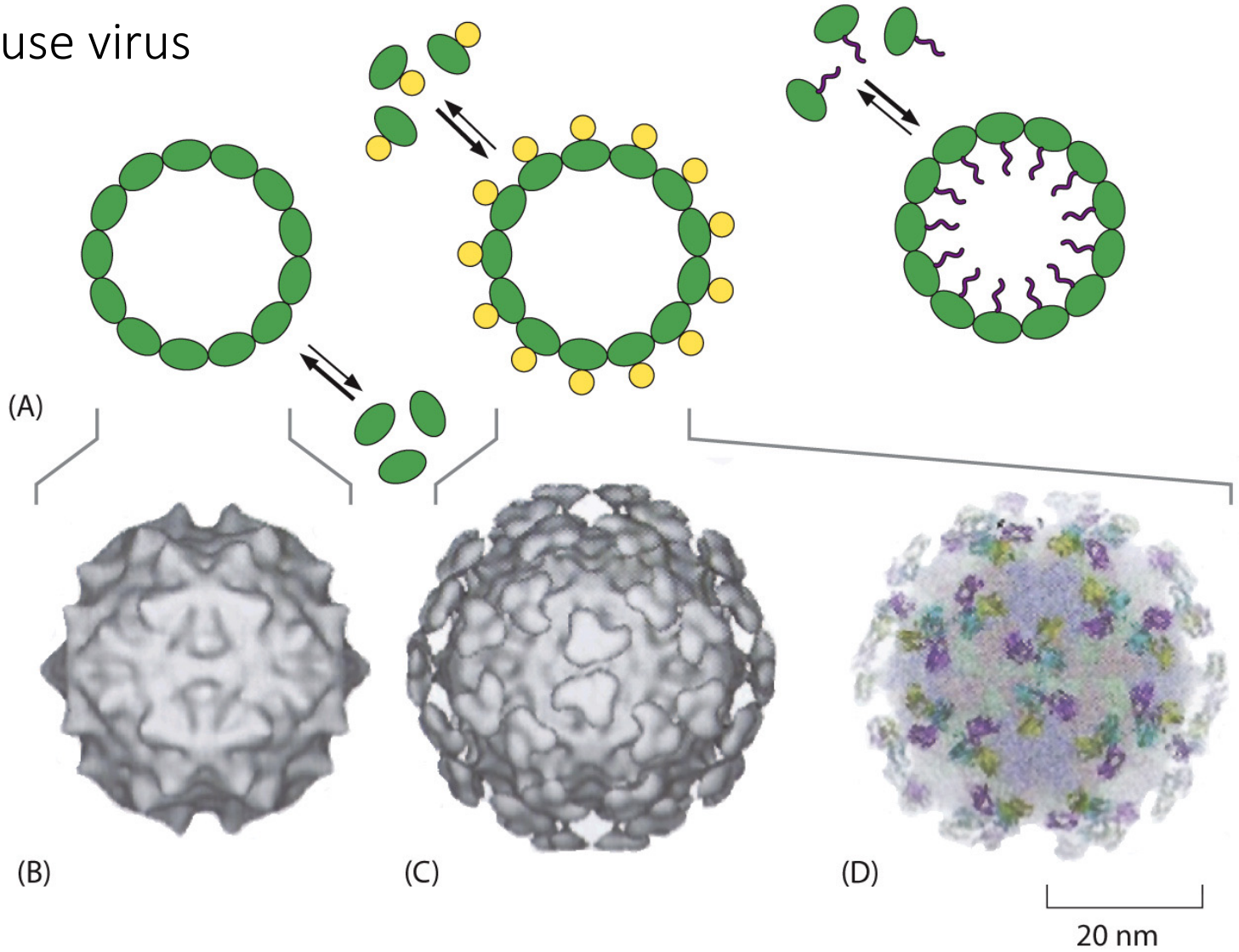
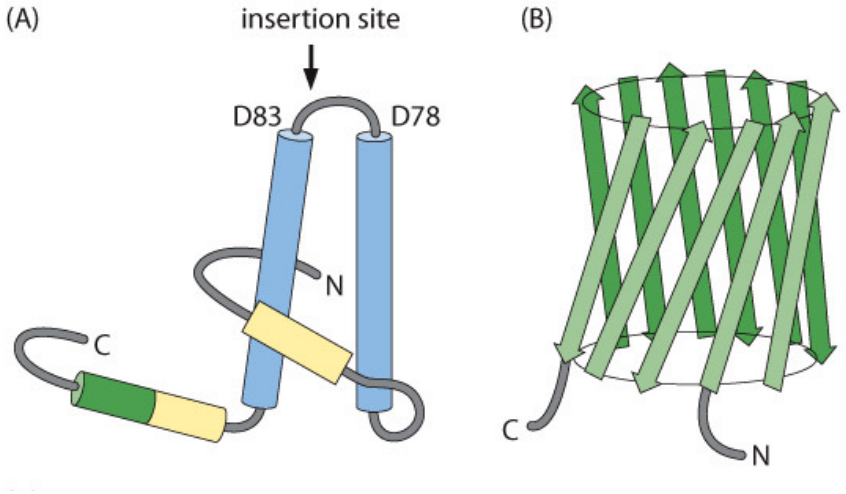


Figure 8.68 Molecular Biology of Assemblies and Machines (© Garland Science 2016)

Display of green fluorescent protein at the tips of HBV capsid spikes



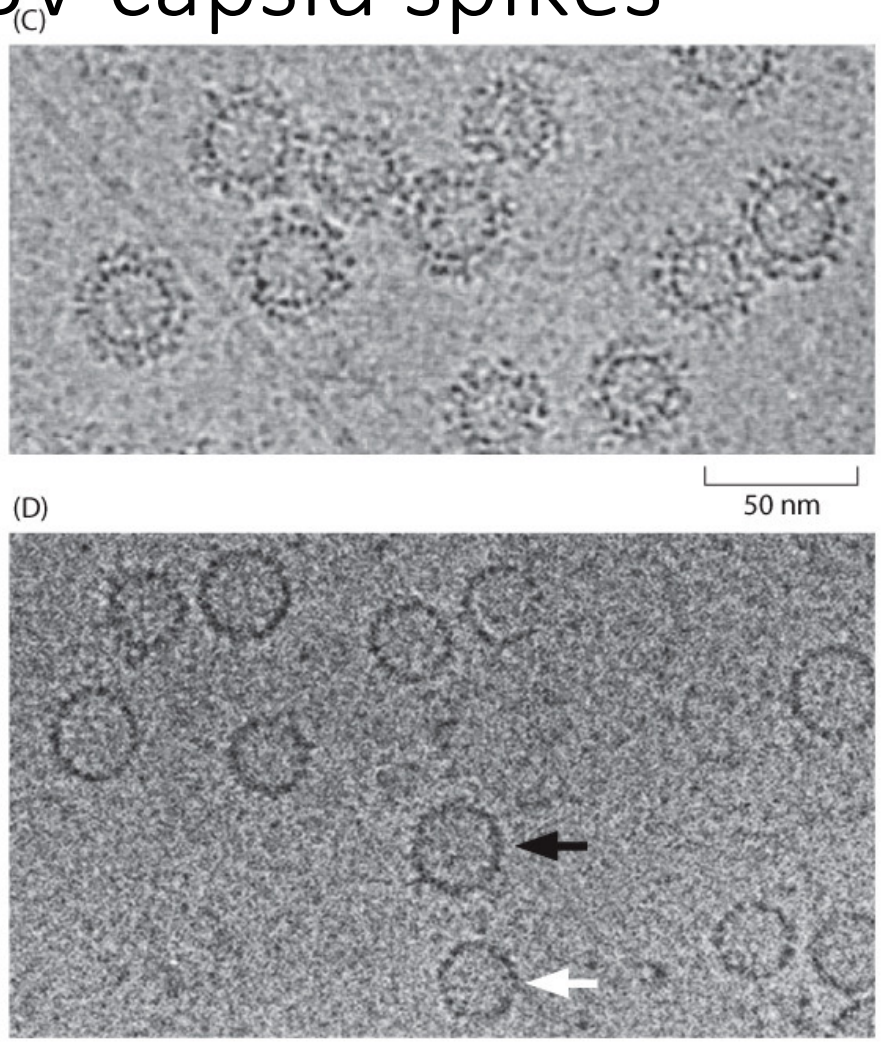


Figure 8.69 Molecular Biology of Assemblies and Machines (© Garland Science 2016)

Generation of protective vaccines

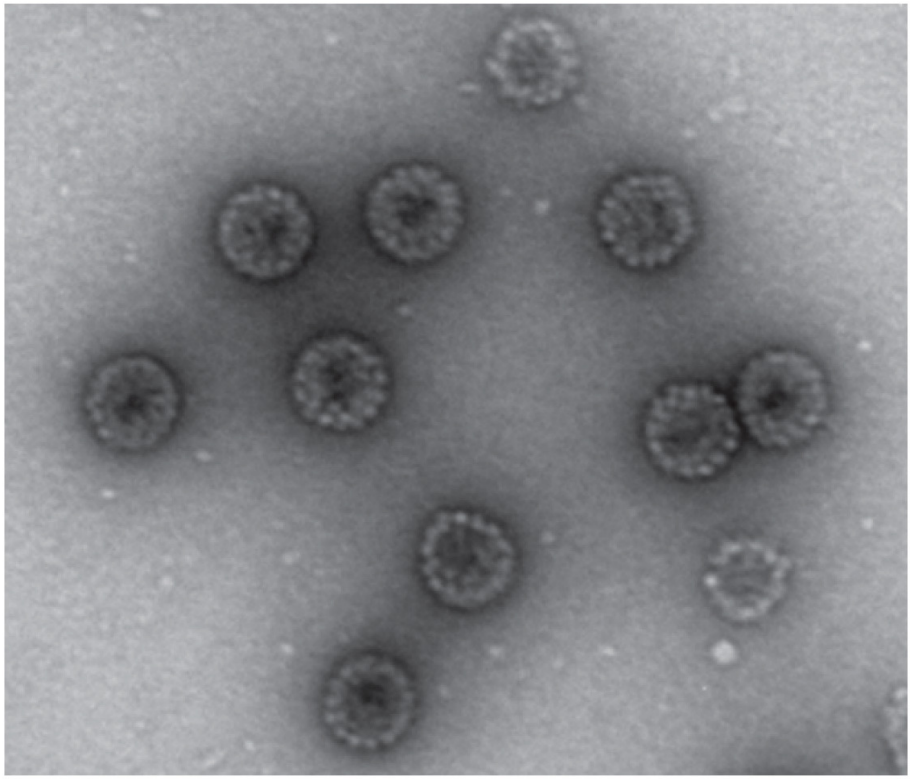


Figure 8.70a Molecular Biology of Assemblies and Machines (© Garland Science 2016)

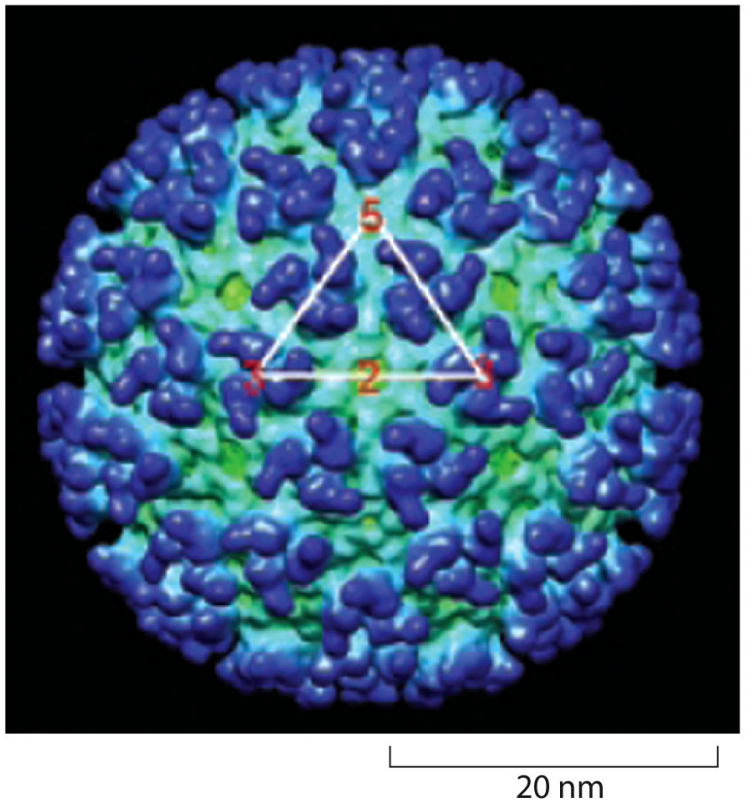


Figure 8.70b Molecular Biology of Assemblies and Machines (© Garland Science 2016)

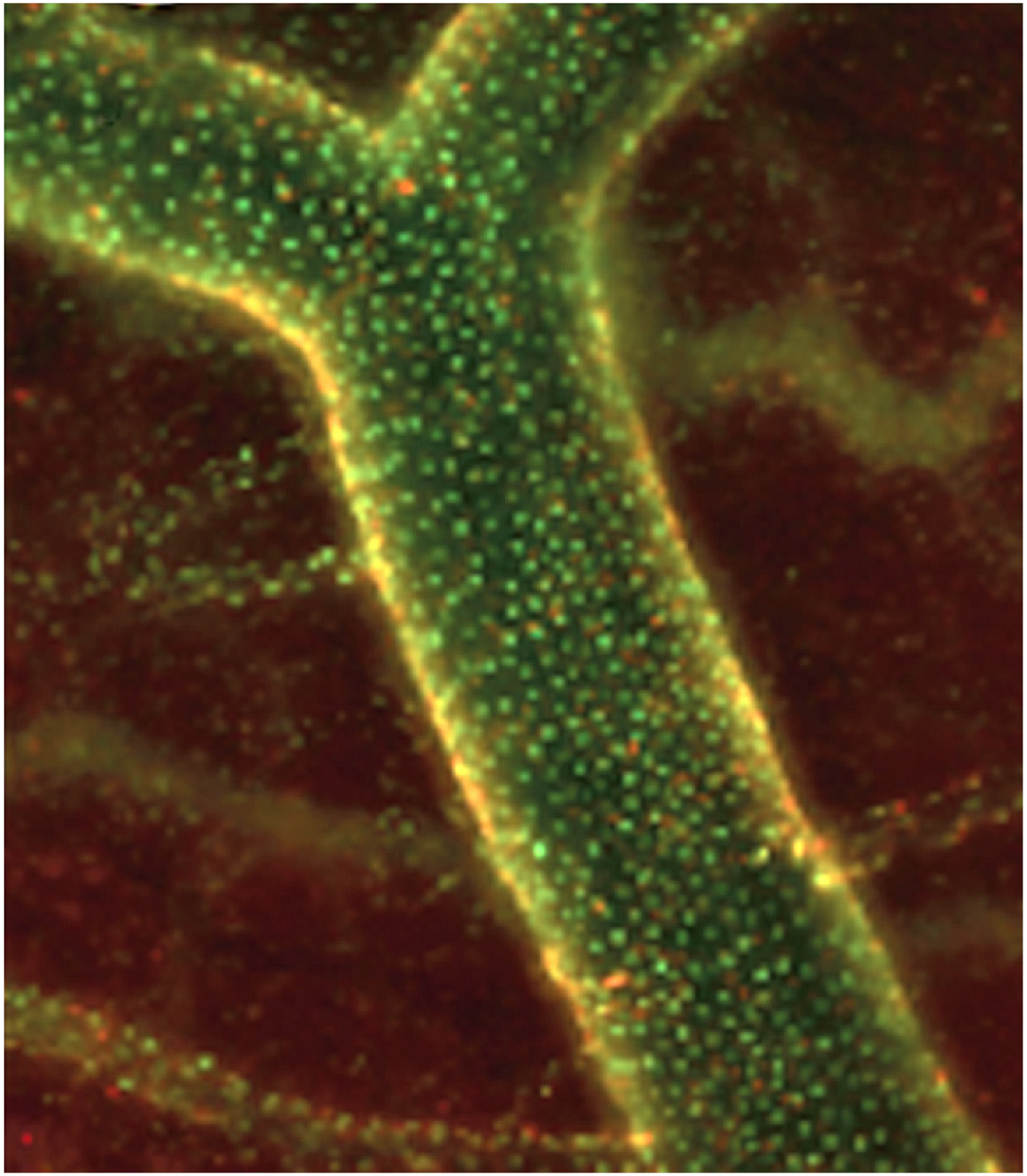


Figure 8.71 Molecular Biology of Assemblies and Machines (© Garland Science 2016)