# The significance of viral entry research

• Simplest biological system for the study of membrane fusion; important for mechanistic understanding

• Many enveloped viruses are medically important

• An understanding of viral entry may lead to new approaches to prevent viral infections

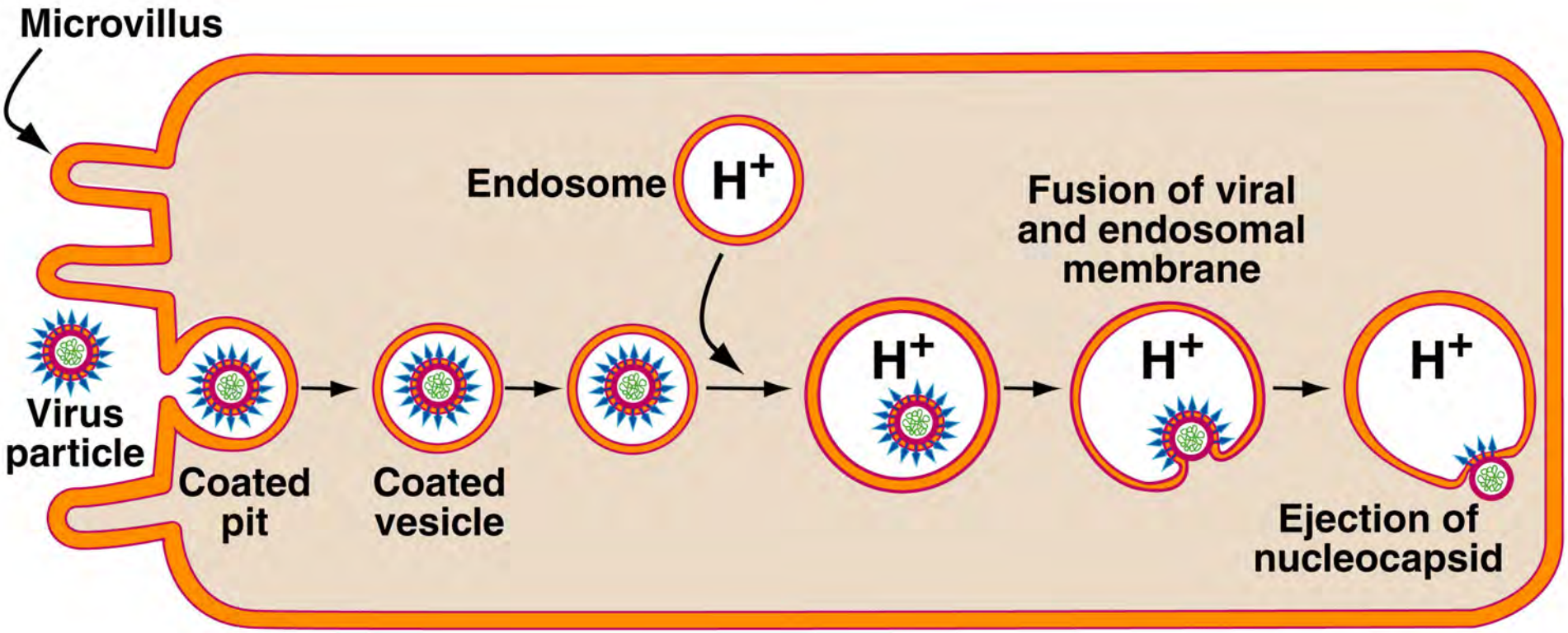
# Influenza virus

• Influenza virus is an orthomyxovirus (with single-stranded RNA viral genome) that causes flu in humans.

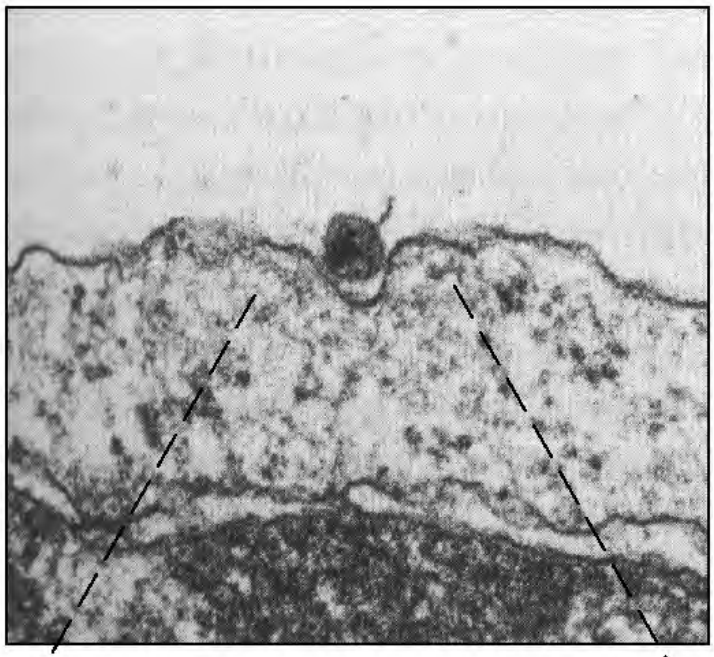
• Virions contain a layer of spikes projecting from the surface. Spikes are made of the viral surface antigens hemagglutinin (HA) and neuraminidase (NA). The subtype of HA and NA is used to classify serotypes of influenza virus (e.g., “H1N1”)

• Influenza pandemic (Spanish flu) in 1918-1919 killed several percent of the world population.

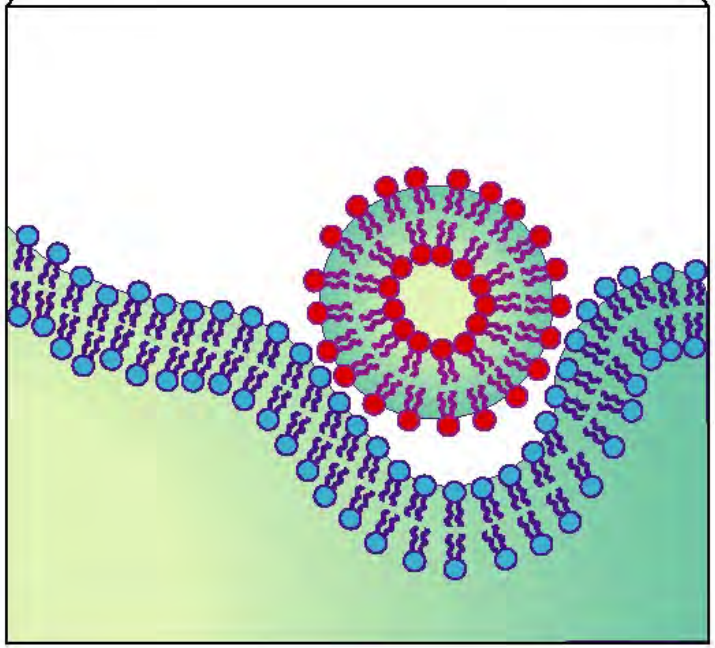
# Influenza entry is triggered by low pH

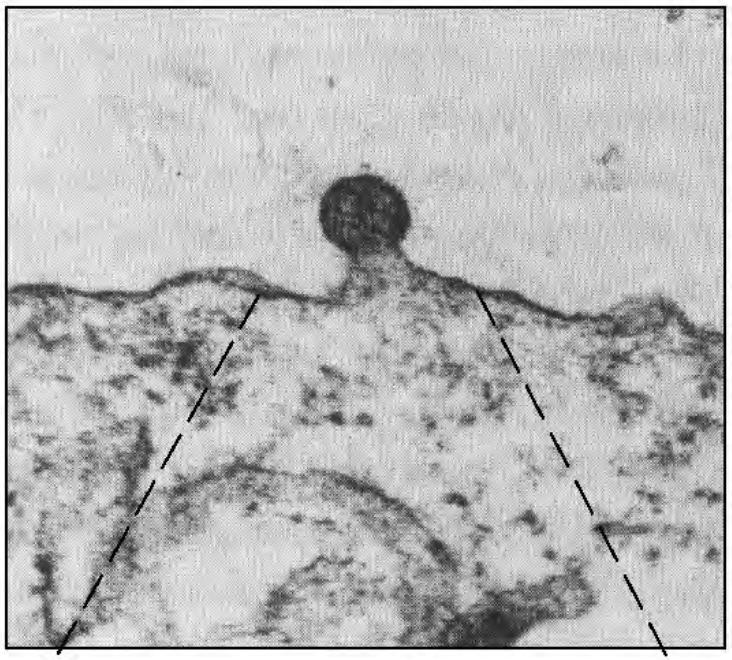


# Fusion of viral and cellular membranes

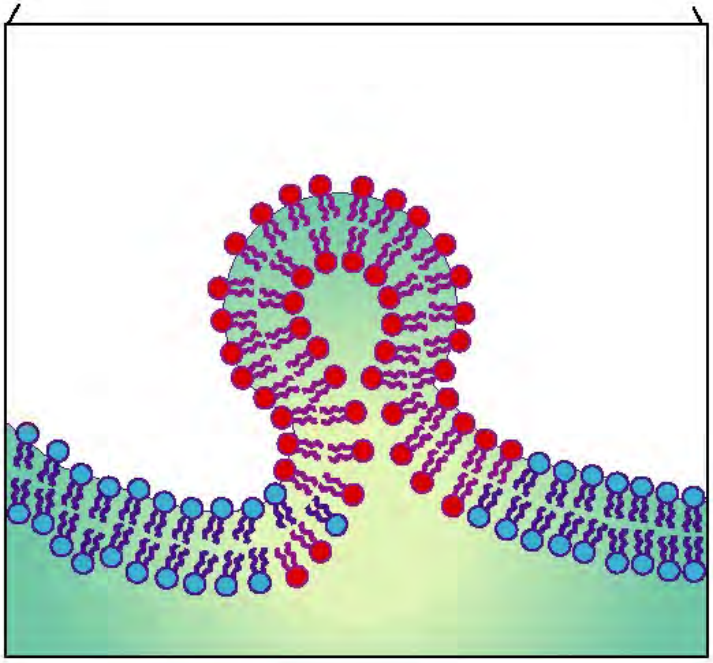


Stein et al. (1987) Cell 49: 664.

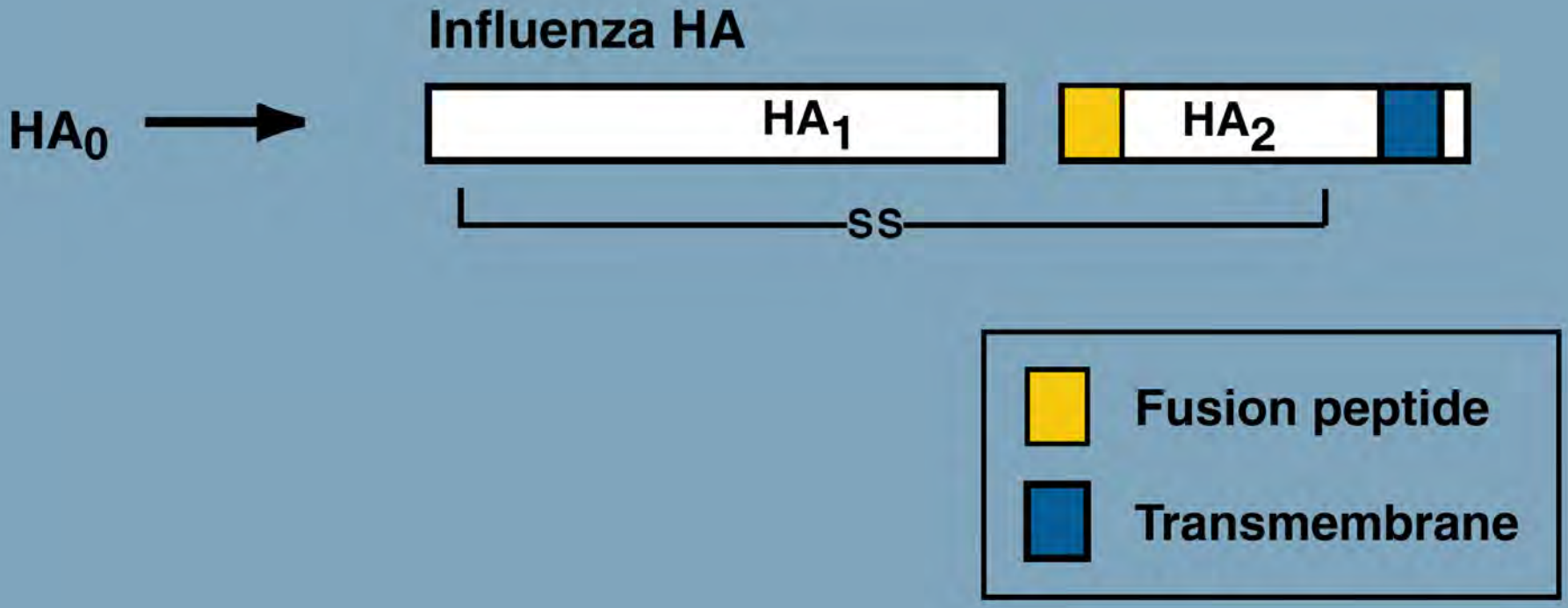




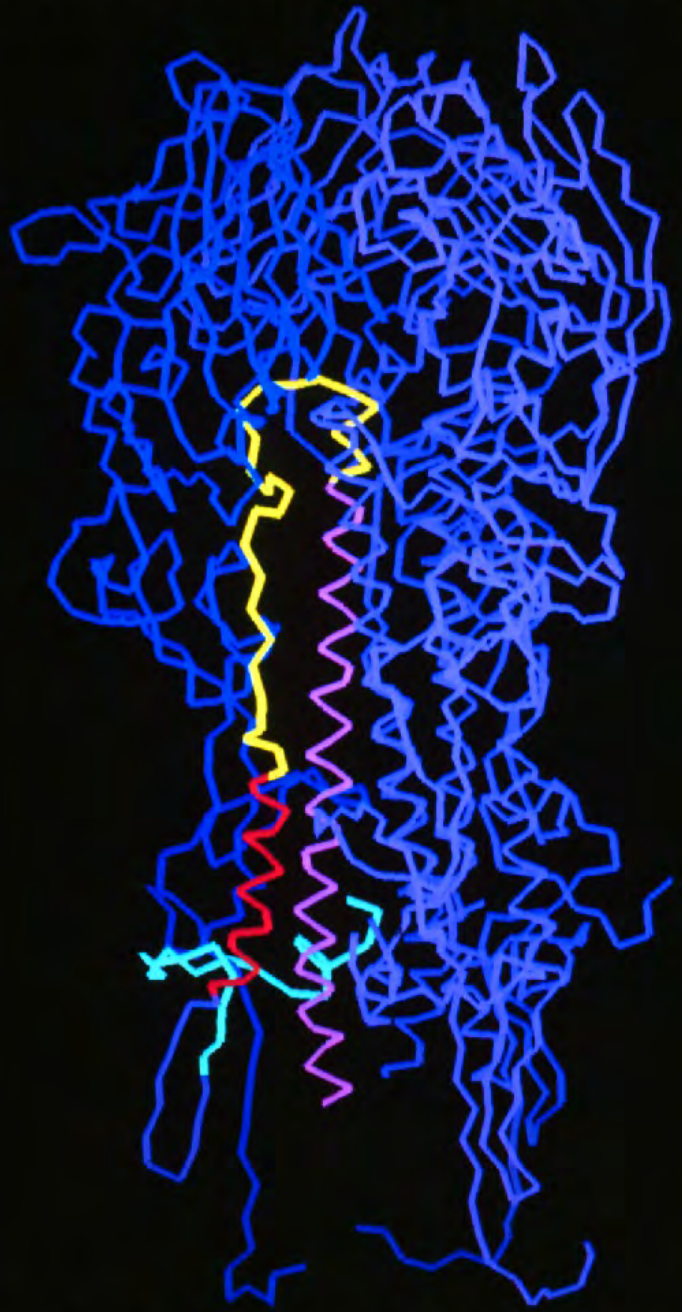
Stein et al. (1987) Cell 49: 664.



# Subunit organization of influenza hemagglutinin

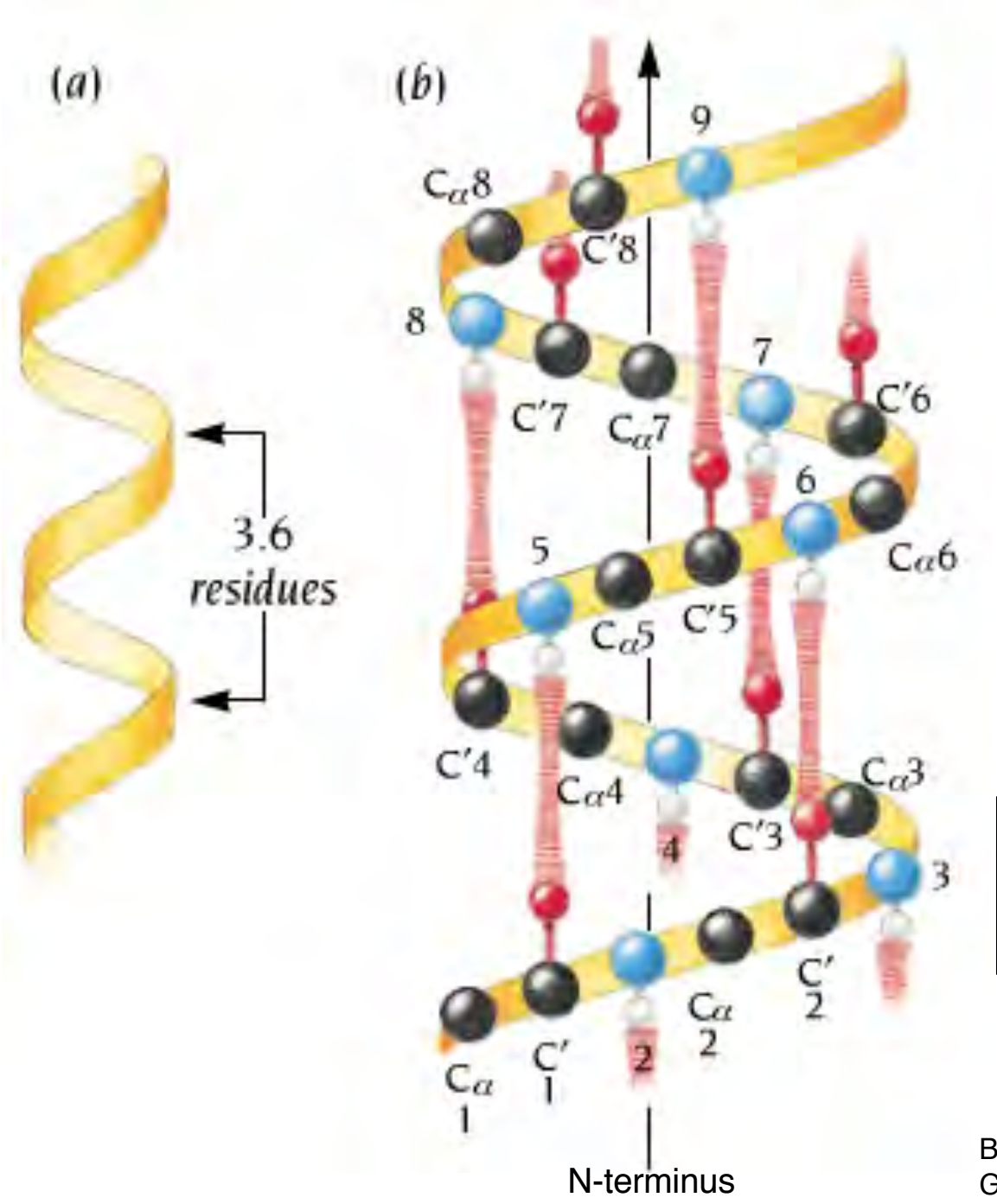


# Native structure of hemagglutinin



Wilson, Skehel & Wiley (1981) Nature 289: 366.

# Structural parameters of an α-helix

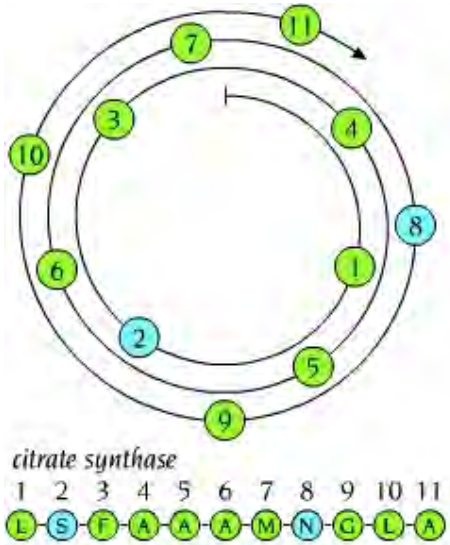



- Right-handed helix
- **3.6 residues per turn** x 1.5Å=5.4Å
- Compare to extended peptide conformation--3.63Å/residue
- Stabilized by hydrogen bonding between carbonyl oxygen of residue N, and nitrogen of residue N+4.

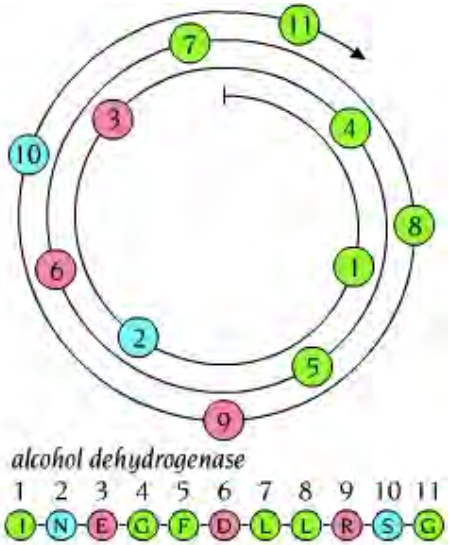
Nitrogen
Carbon
Oxygen

Branden & Tooze,
Garland Publishing Inc

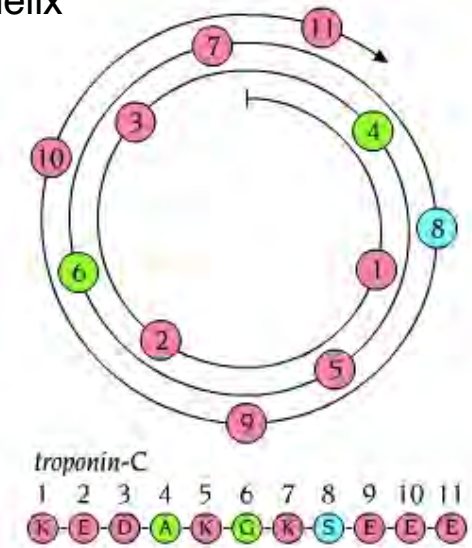
# Helical wheel representation of α-helices




Hydrophobic helix




Amphipathic helix




Polar helix



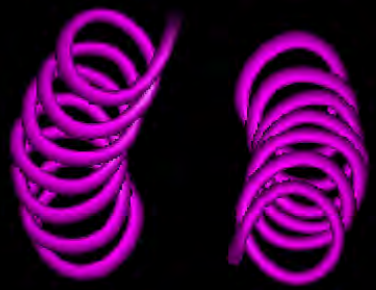
For α-helix:
One residue plotted per 100˚ (360˚/3.6 residues) on the circle.

## Yeast GCN4 contains a prototypical coiled coil

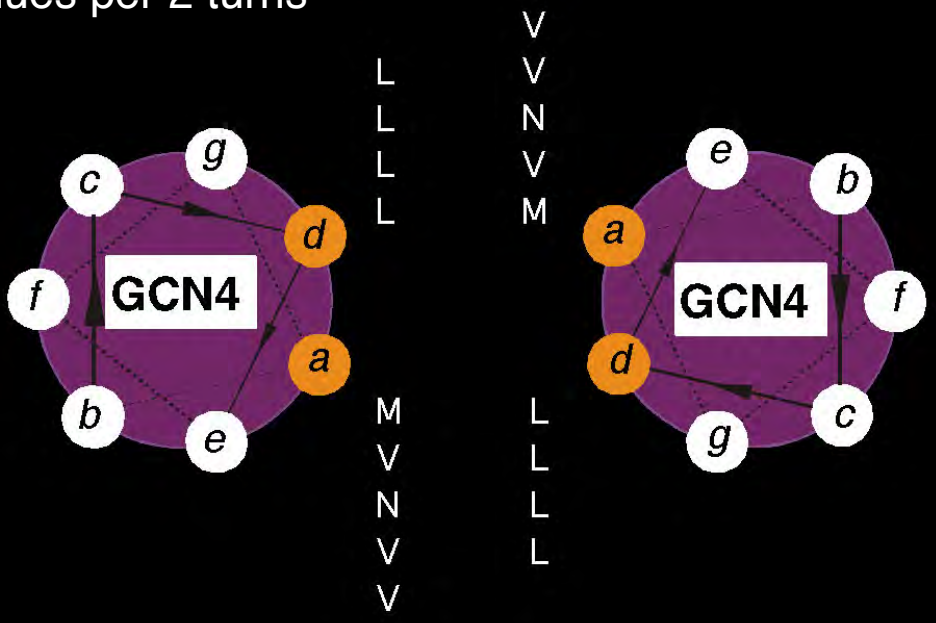
**M** K Q **L** E D K **V** E E **L** L S K **N** Y H **L** E N E **V** A R **L** K K L **V** . . .
**a** b c **d** e f g **a** b c **d** e f g **a** b c **d** e f g **a** b c **d** e f g **a** . . .

(1) 4,3 hydrophobic repeat
(2) fibrous proteins, transcription factors, viral envelope proteins
(3) oligomerization interface

# Coiled coiled are stabilized by hydrophobic packing in the core *a* and *d* positions

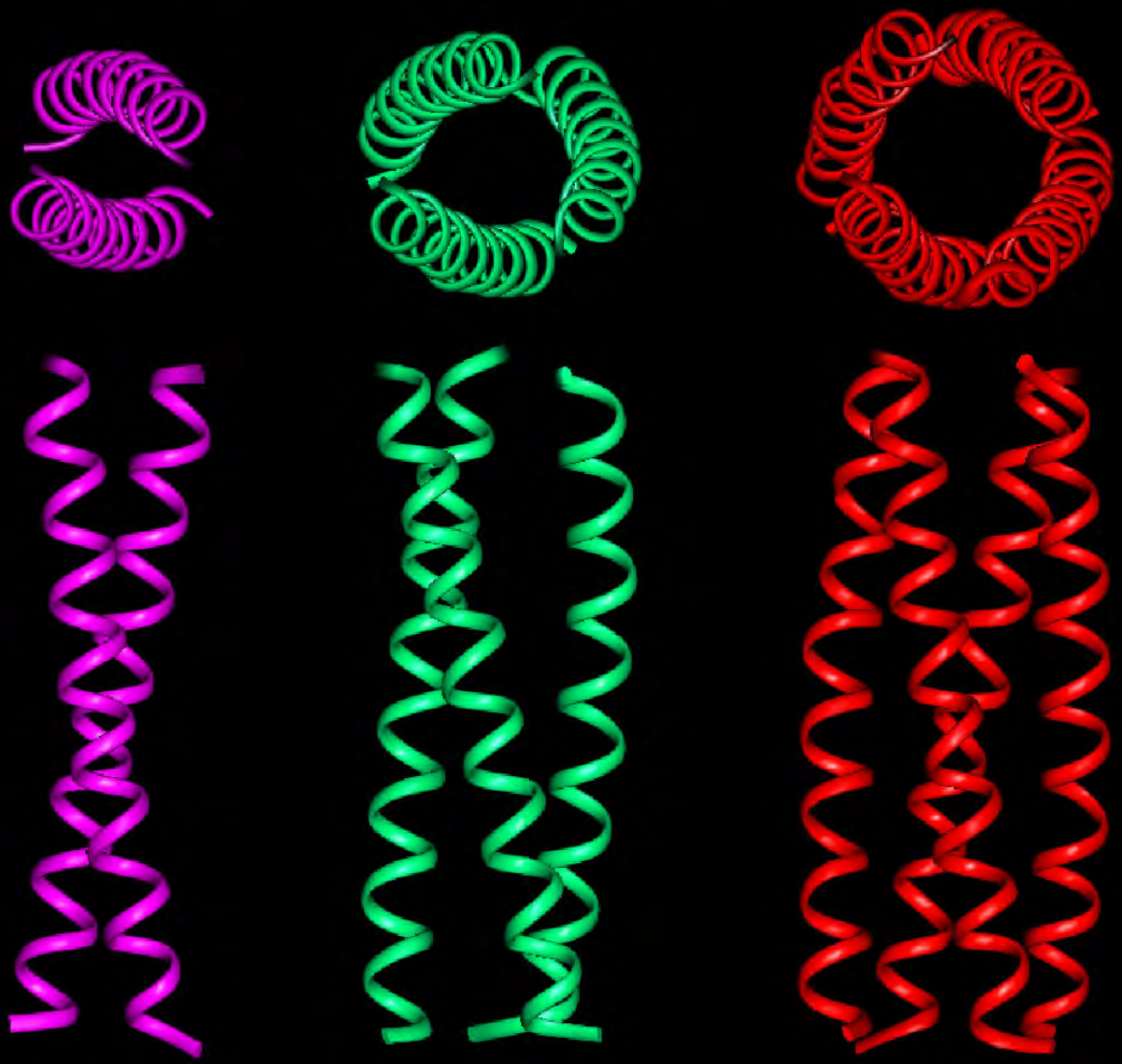


Coiled coil: 7 residues per 2 turns



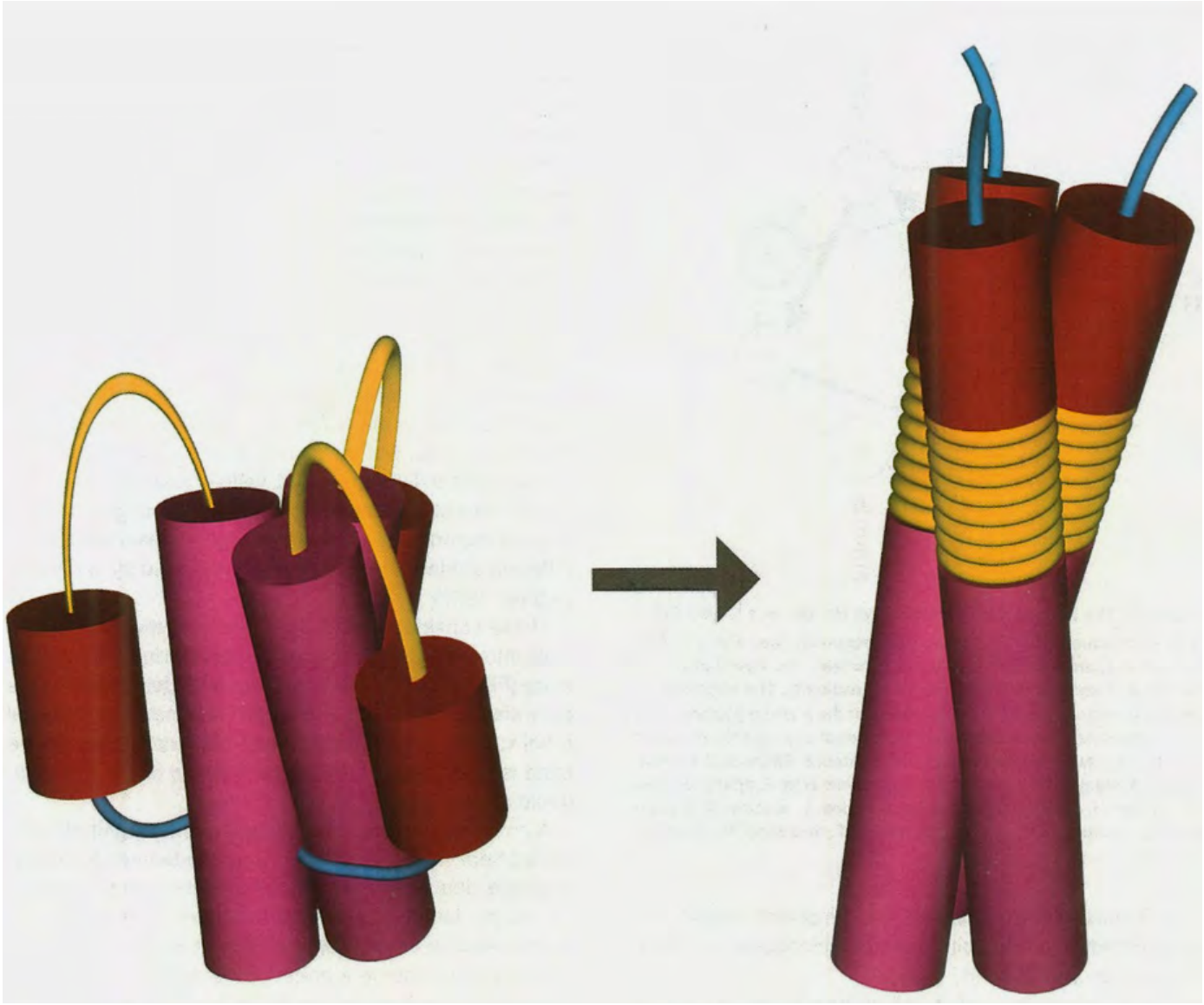

O'Shea et al. (1991) Science 254:539

# 2, 3, and 4-stranded coiled coils



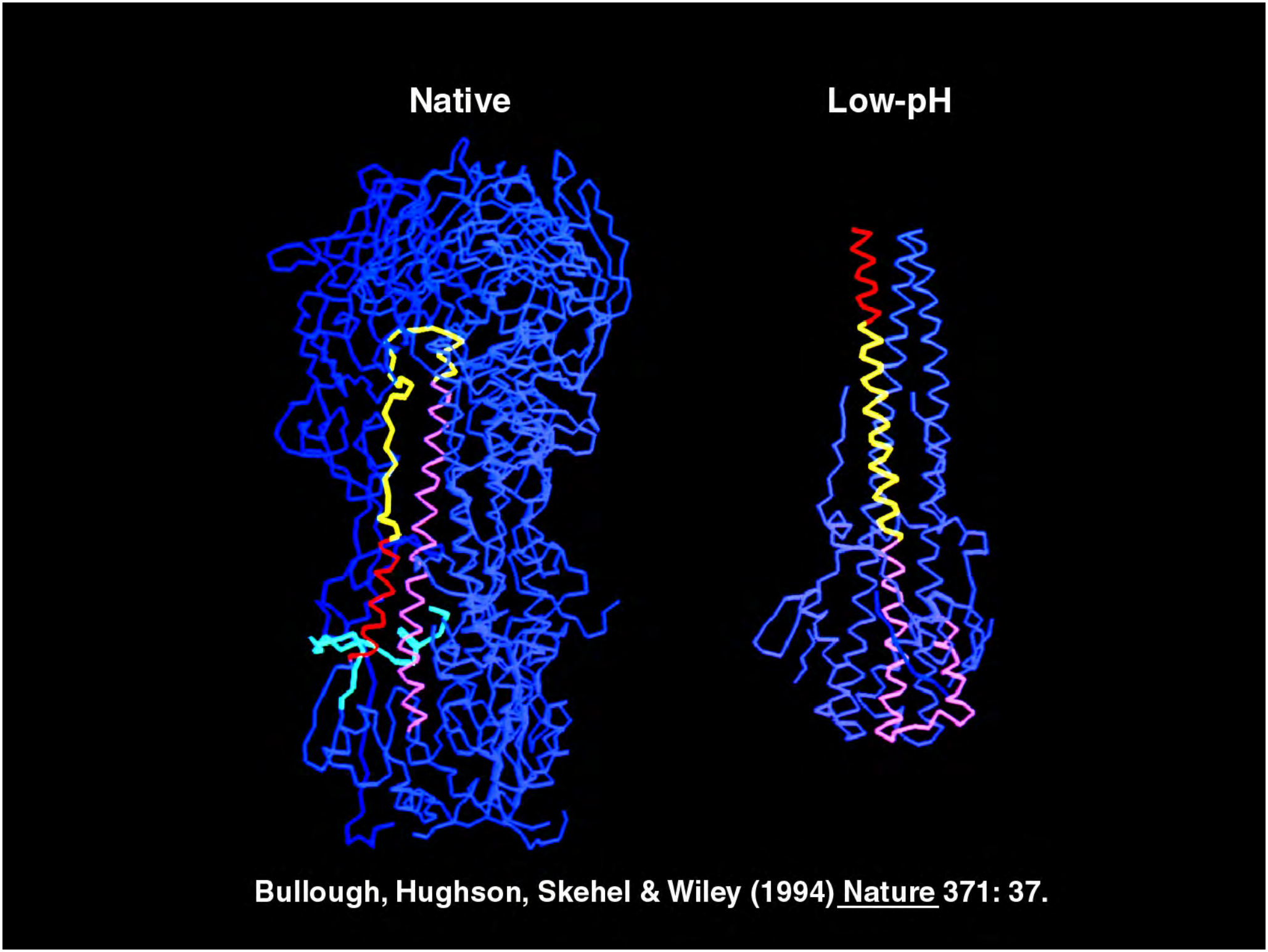
Harbury et al. (1993) Science 262:1401

# A spring-loaded model for HA2 conformational change



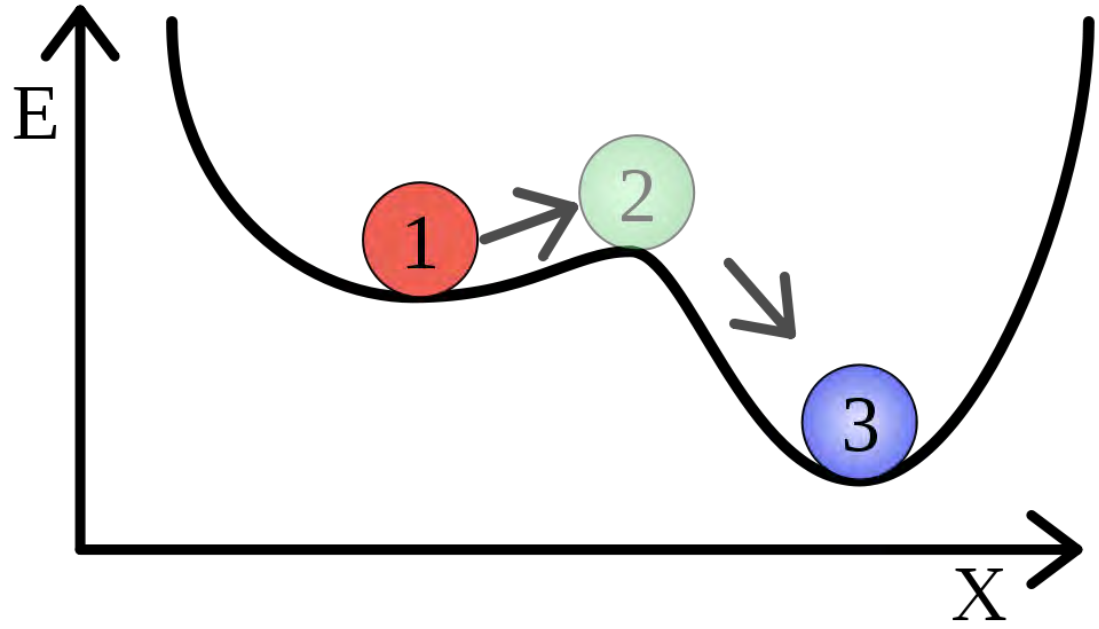
- Native state of HA2 on left, with blue fusion peptide buried.
- In fusogenic state, fusion peptide is released due to a loop-to-helix transition.
- Fusion peptide relocated ~100 Å towards target membrane.

Carr & Kim (1993) Cell




Bullough, Hughson, Skehel & Wiley (1994) Nature 371: 37.

# Native HA2 is a metastable state

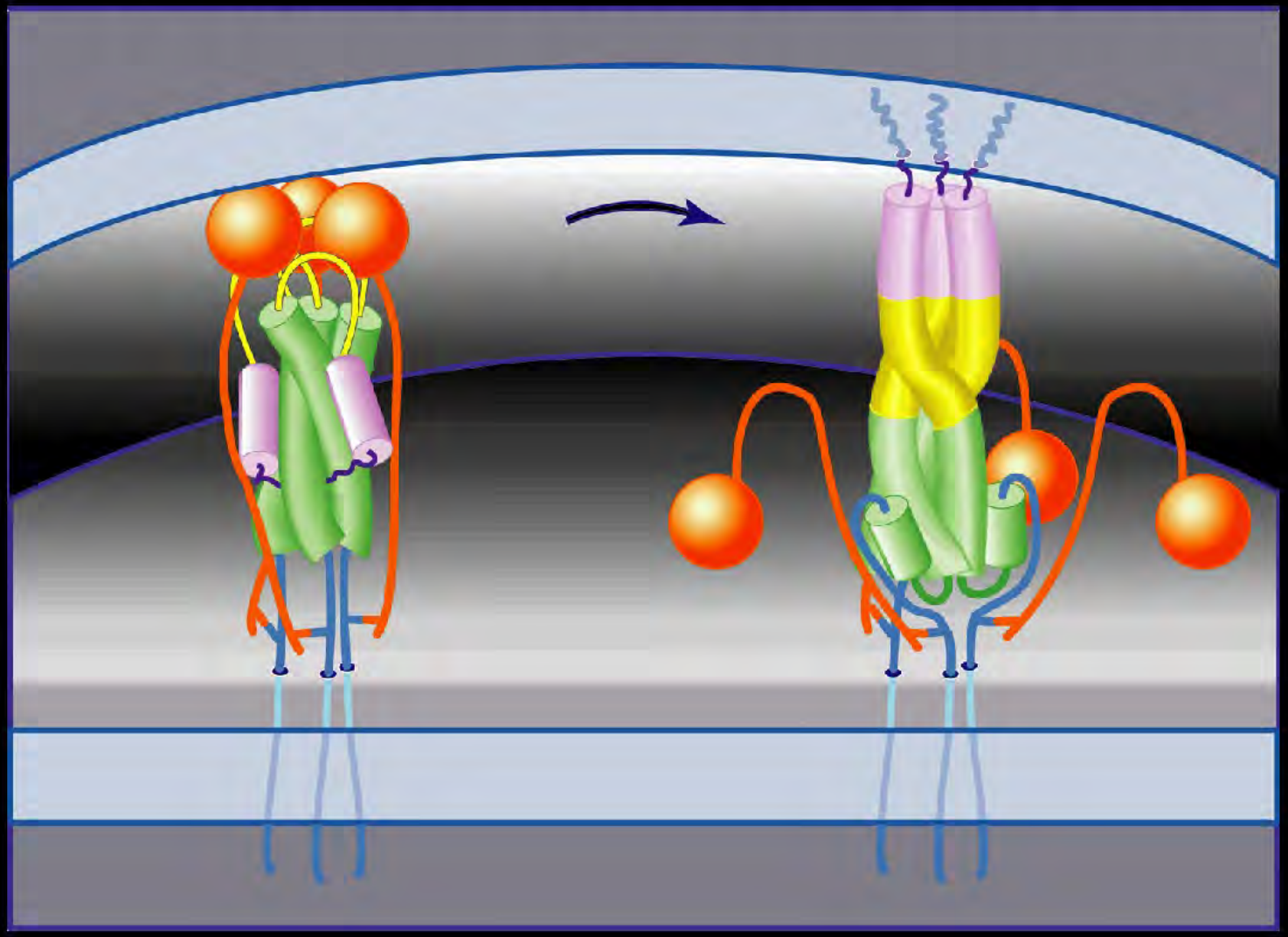



www.wikipedia.org

**Metastability:**

- Native HA2 is produced in a metastable state (1), stabilized by interactions with HA1
- Low pH triggers a conformational change to the fusion-active state (3), a thermodynamically more stable state.

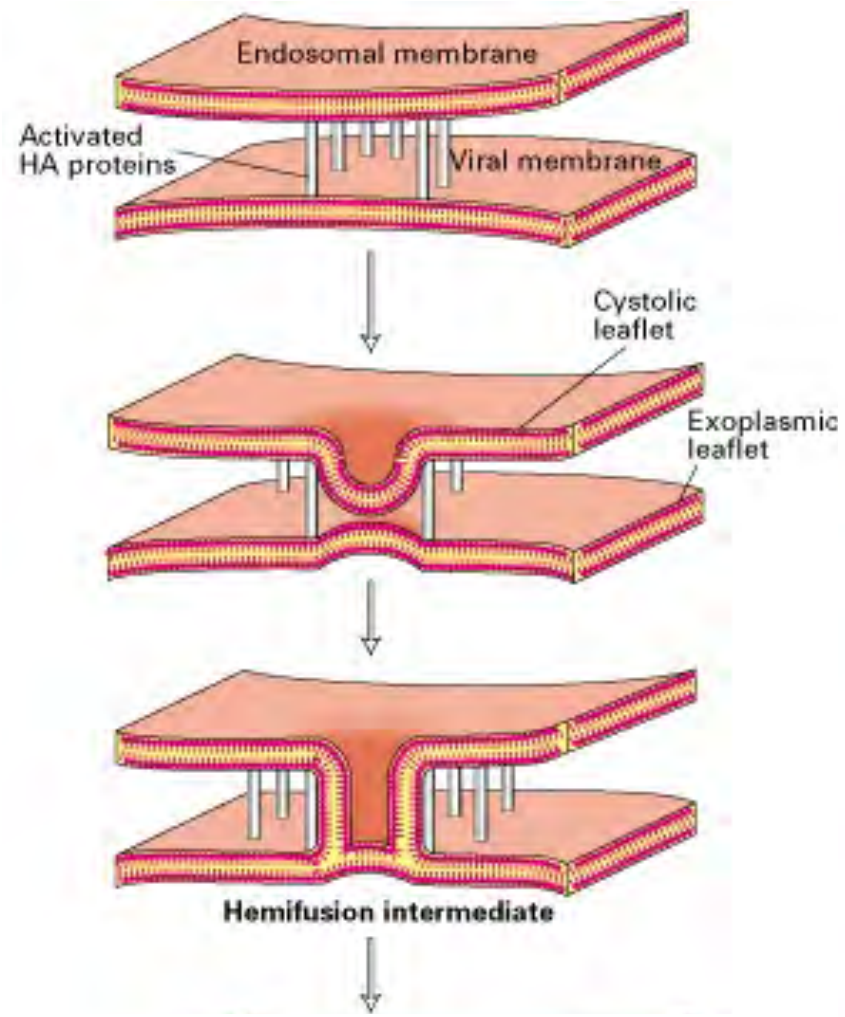
# Spring-loaded mechanism for influenza membrane fusion

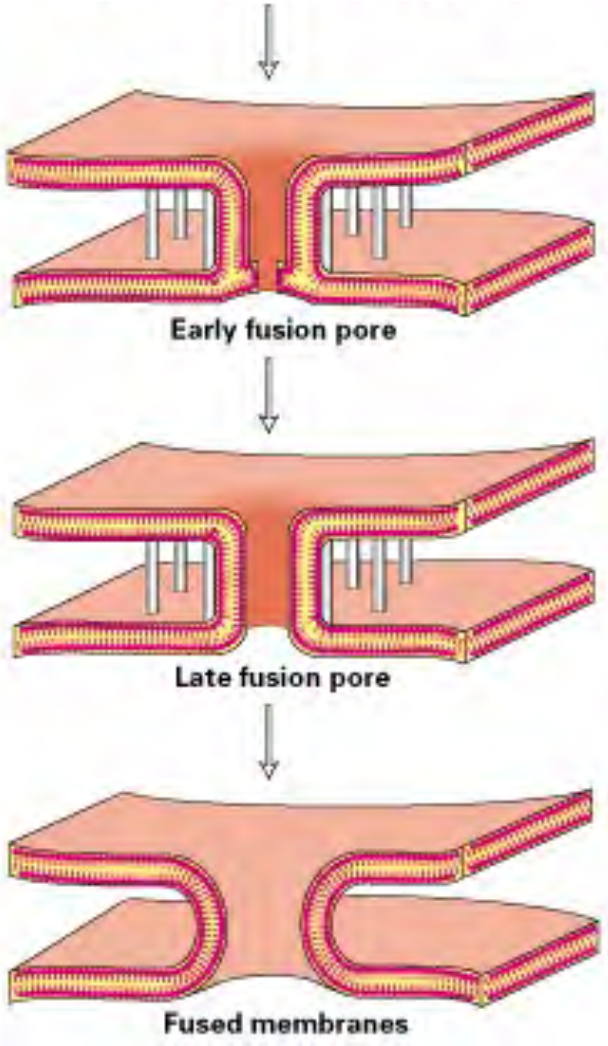


Jodi M. Harris

Carr & Kim (1993) Cell 73, 823.
Bullough, Hughson, Skehel & Wiley (1994) Nature 371, 37.

# Virus membrane fusion may proceed through a hemifusion intermediate

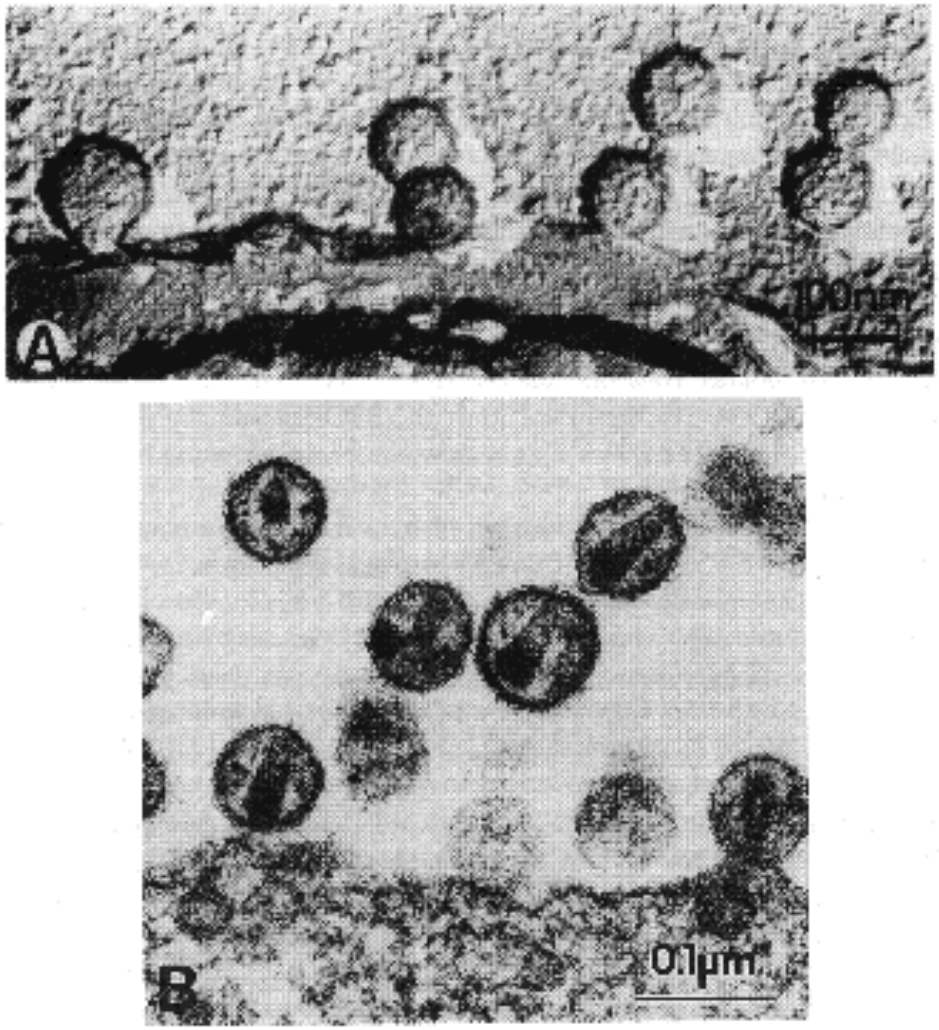



The transmembrane segment of HA may be important for full fusion.

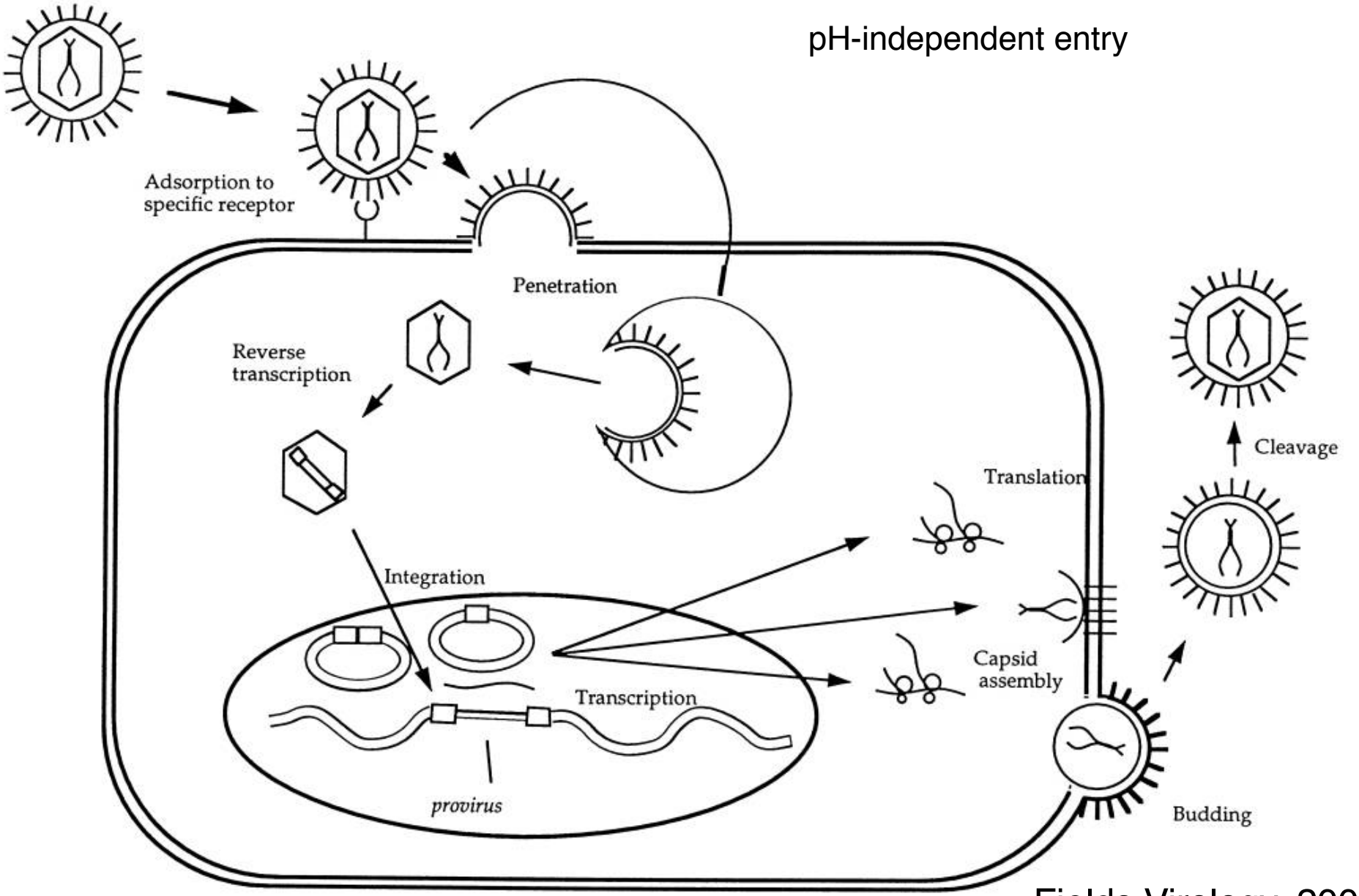
Lodish et al, 2000

# HIV virions budding from a human cell



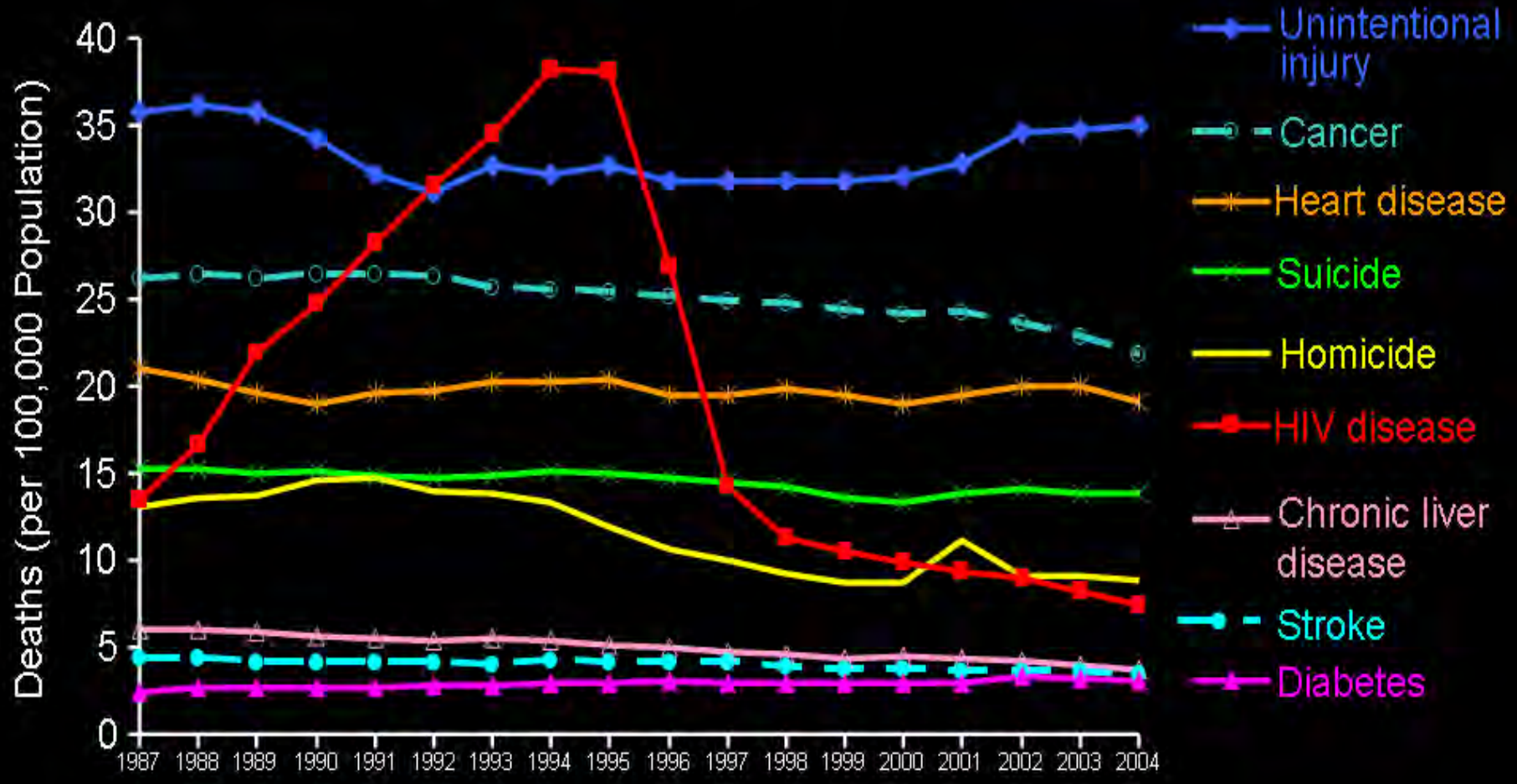

R. Munn in Levy, 1994

# Retroviral lifecycle



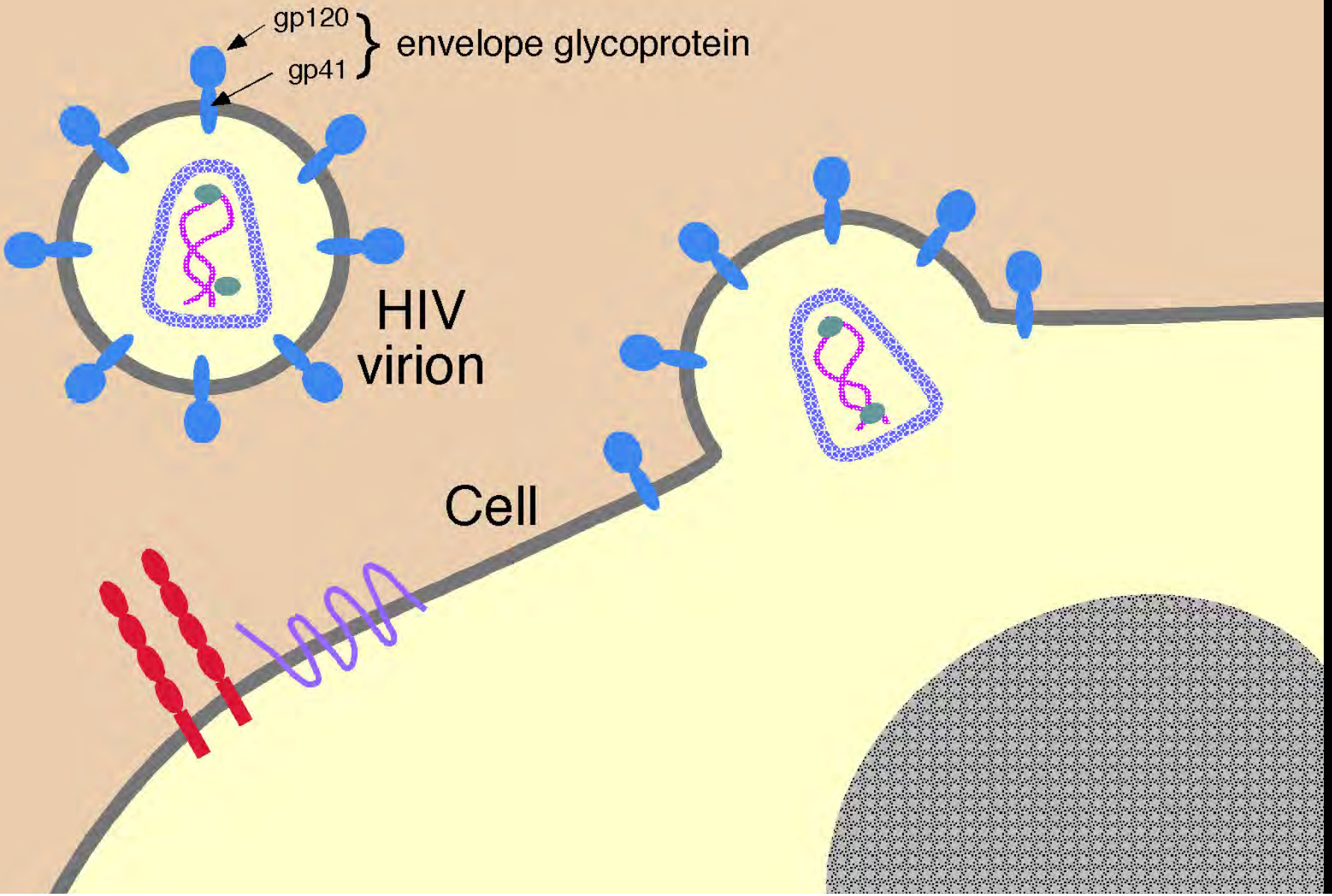

Fields Virology, 2001

# Trends in Annual Rates of Death due to the 9 Leading Causes among Persons 25–44 Years Old, United States, 1987–2004

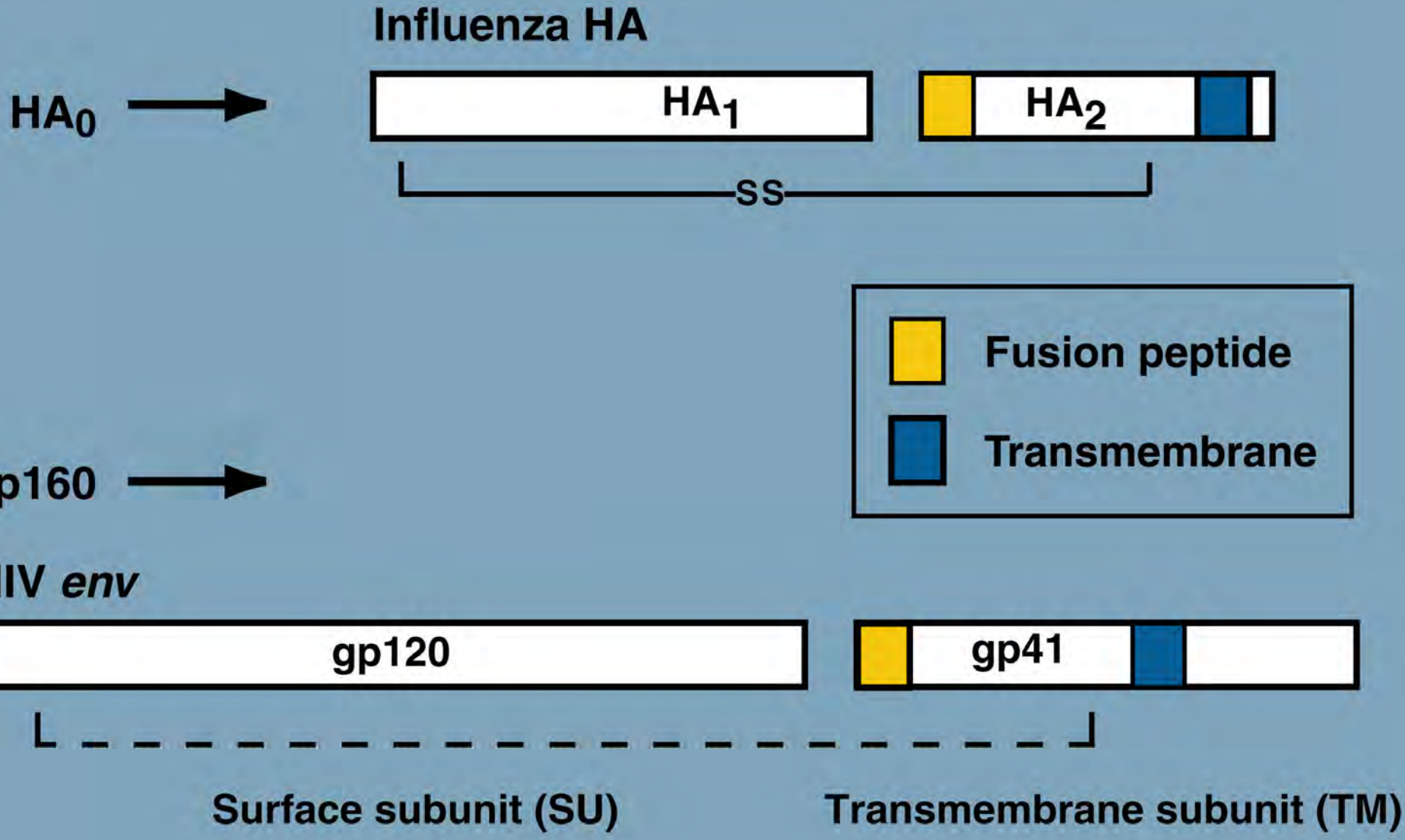



Note: For comparison with data for 1999 and later years, data for 1987–1998 were modified to account for *ICD-10* rules instead of *ICD-9* rules.

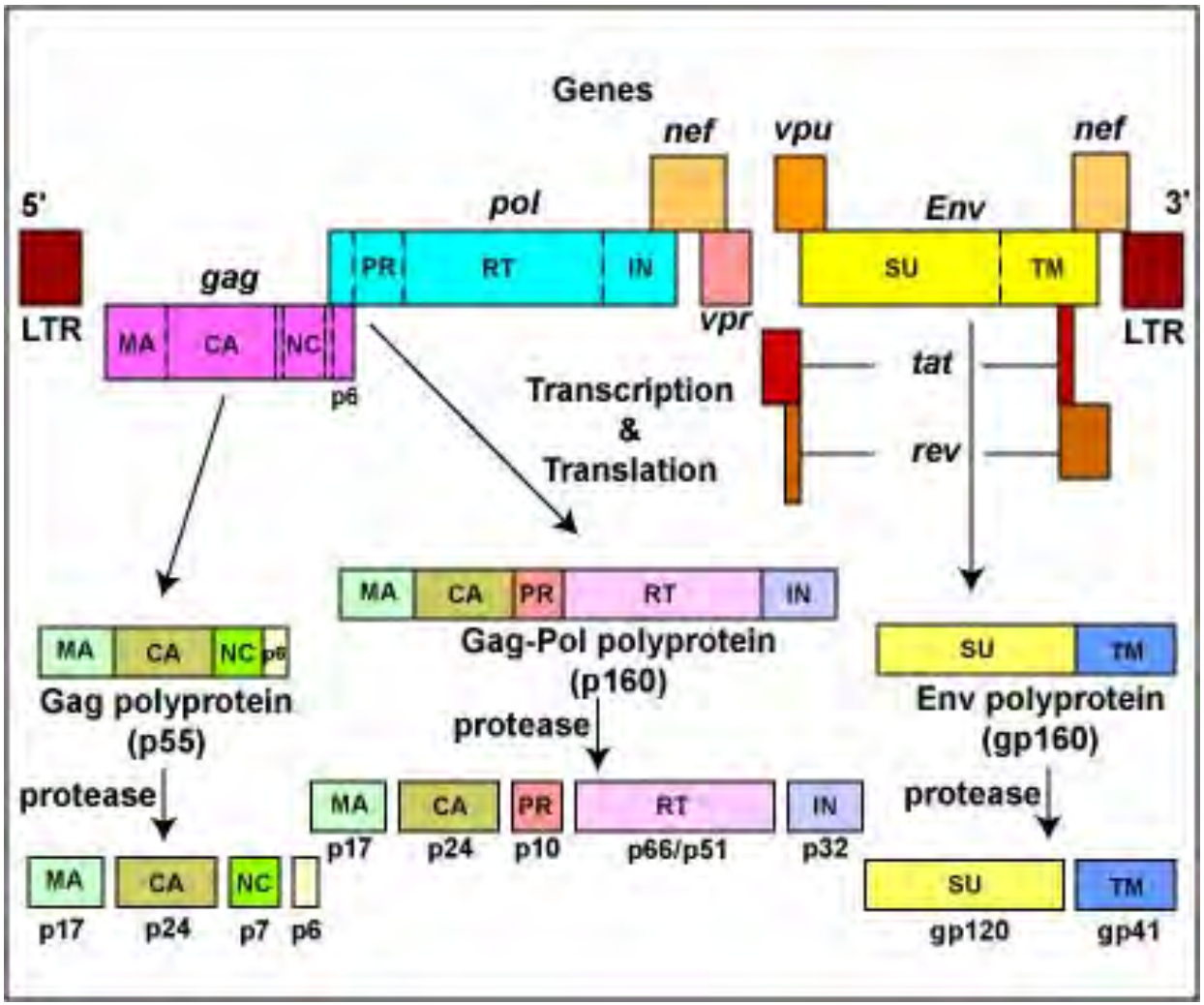
# Membrane fusion activity of HIV gp41 is triggered by receptor binding



# Subunit organization of fusion glycoproteins



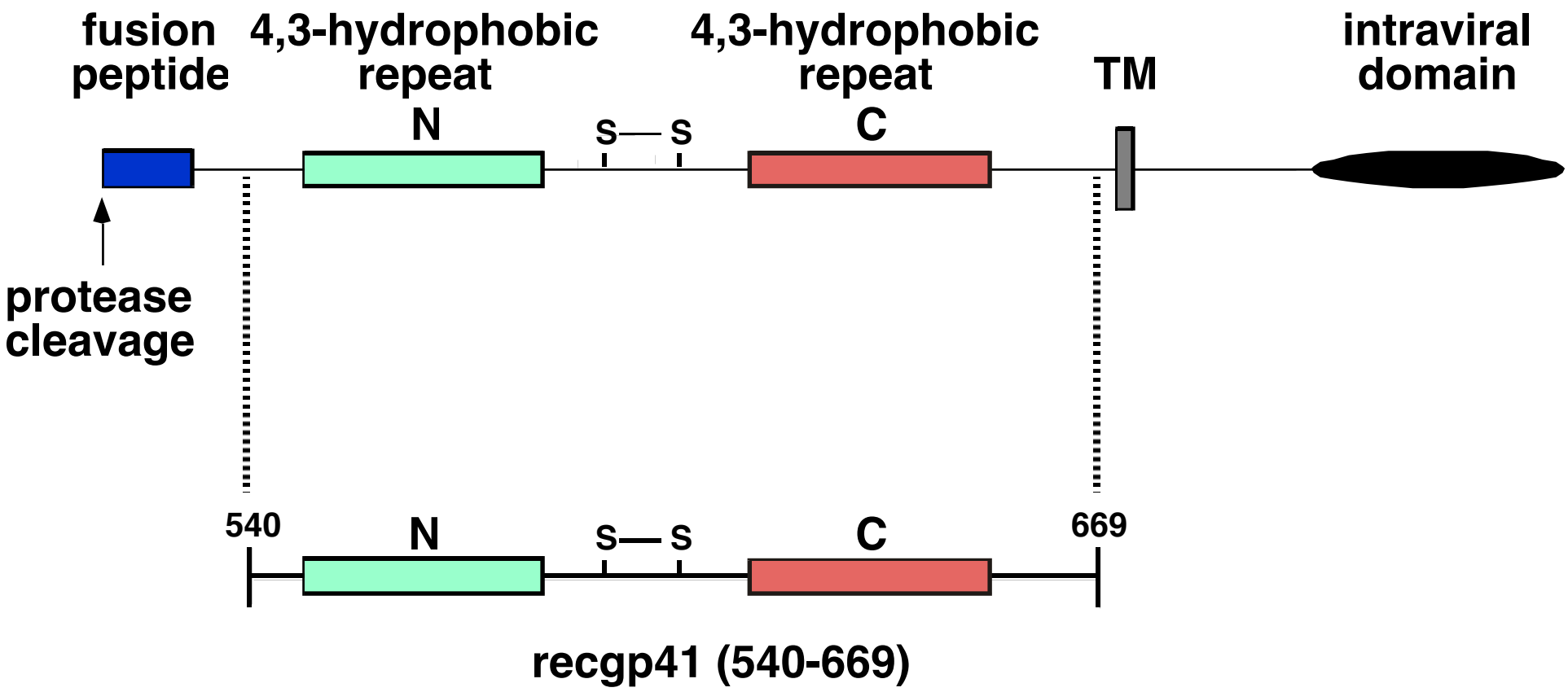
# HIV-1 protease versus furin-type protease




HIV-1 gp160 is cleaved by a (cellular) furin-type protease into gp120 and gp41.

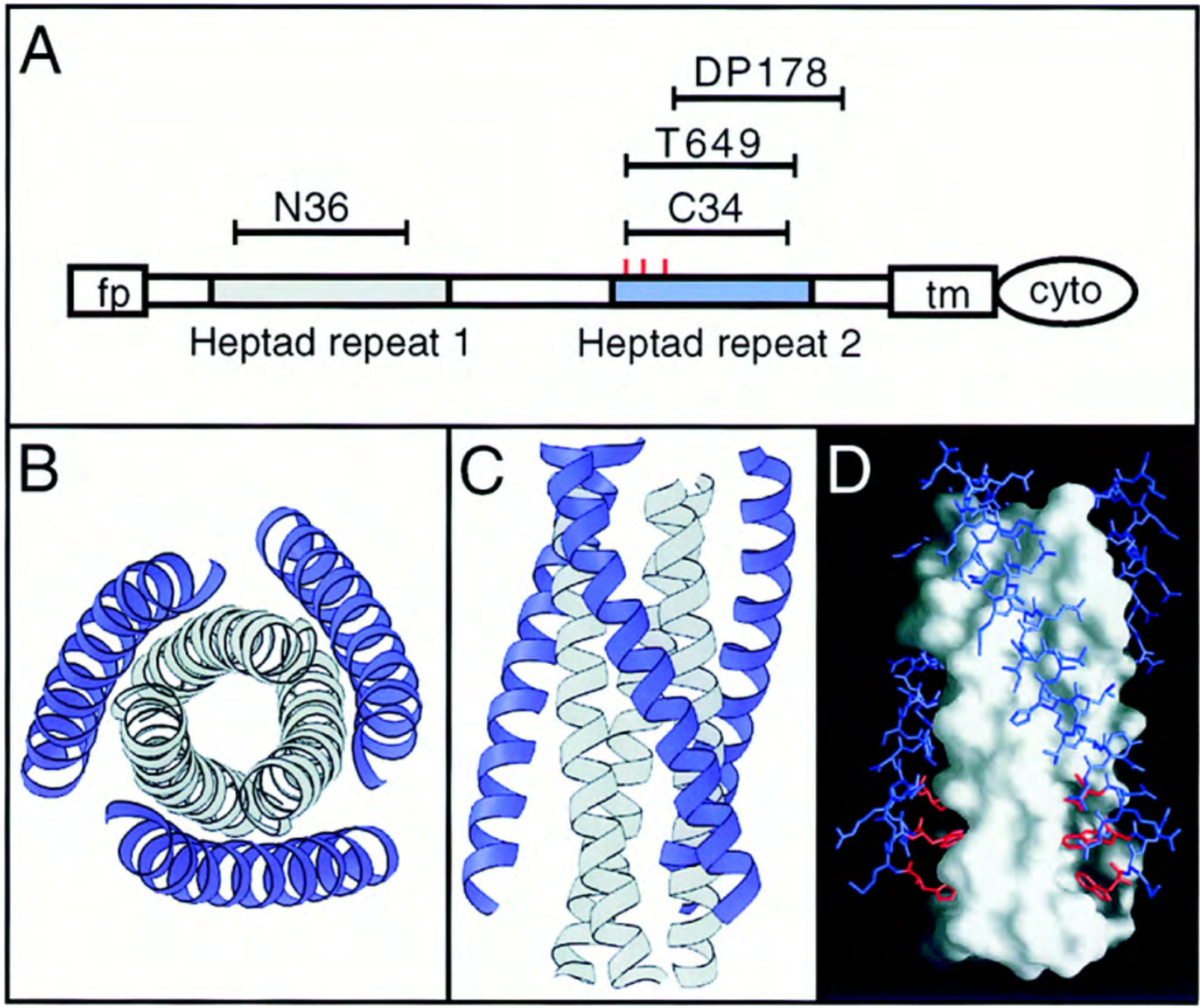
Gag and Gag-Pol polyproteins are cleaved by the HIV-1 protease, which is targeted by protease inhibitors.

# Protein dissection of the ectodomain of HIV gp41



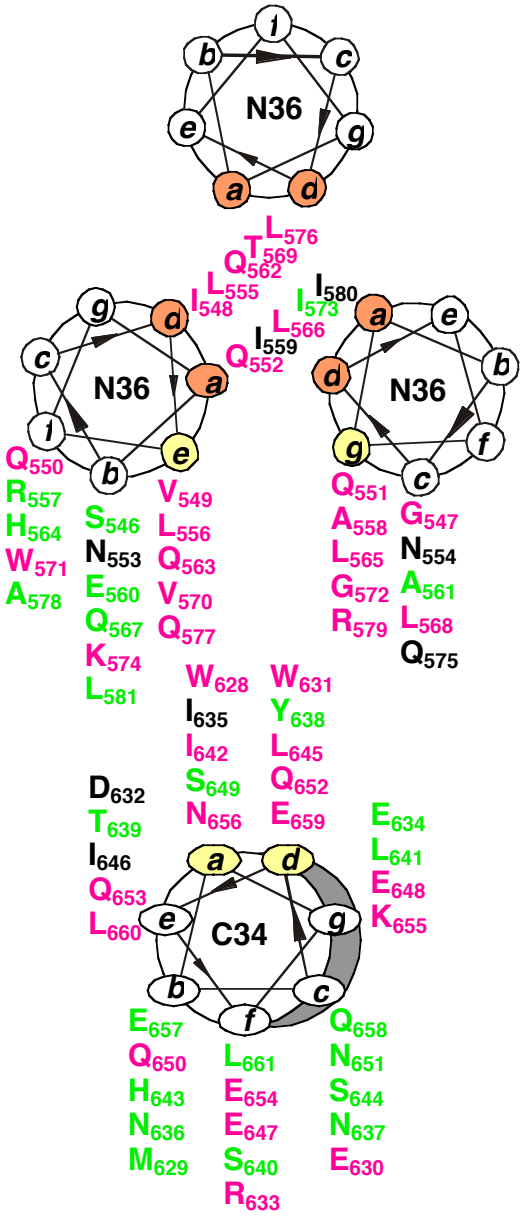

Lu, Blacklow & Kim (1995) Nat. Struct. Biol. 2: 1075

# The gp41 ectodomain is a 6-helix bundle

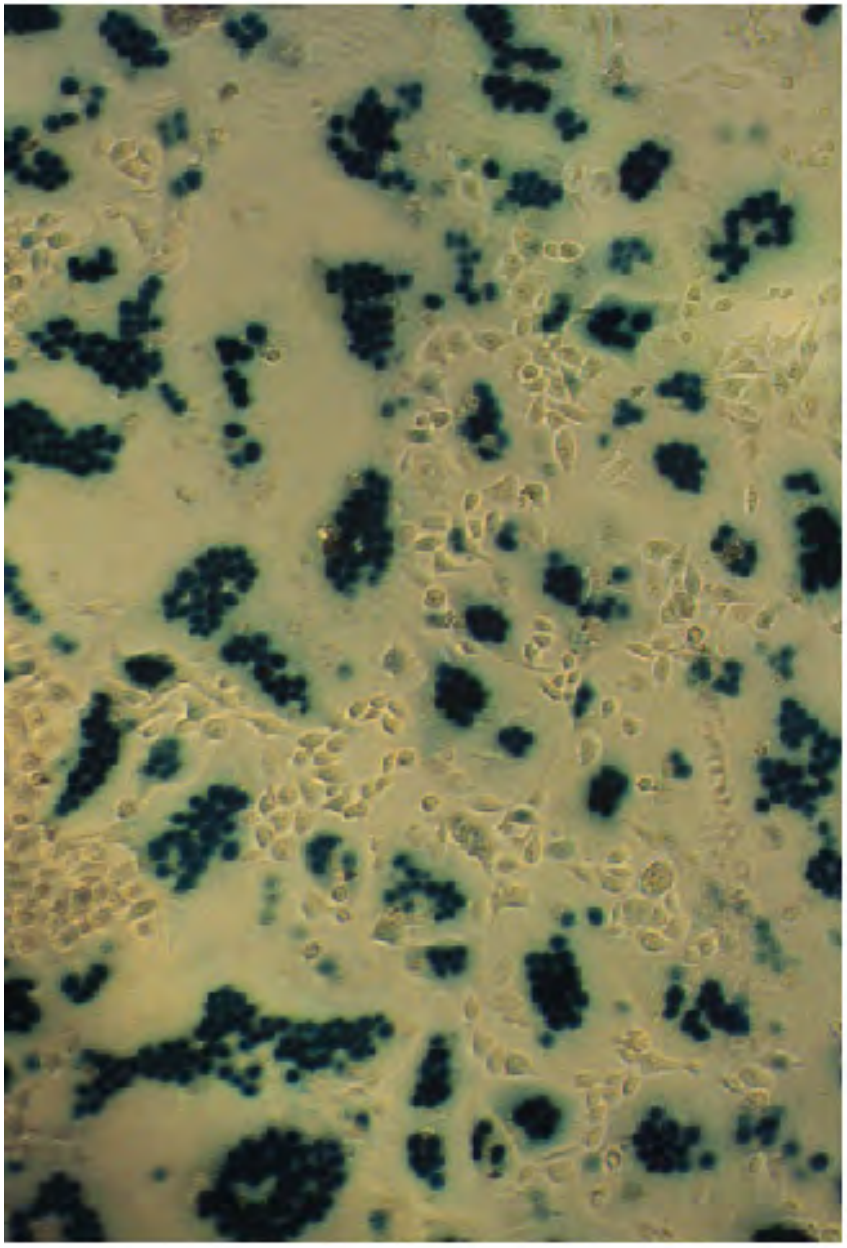



Chan et al (1997) Cell 89:263

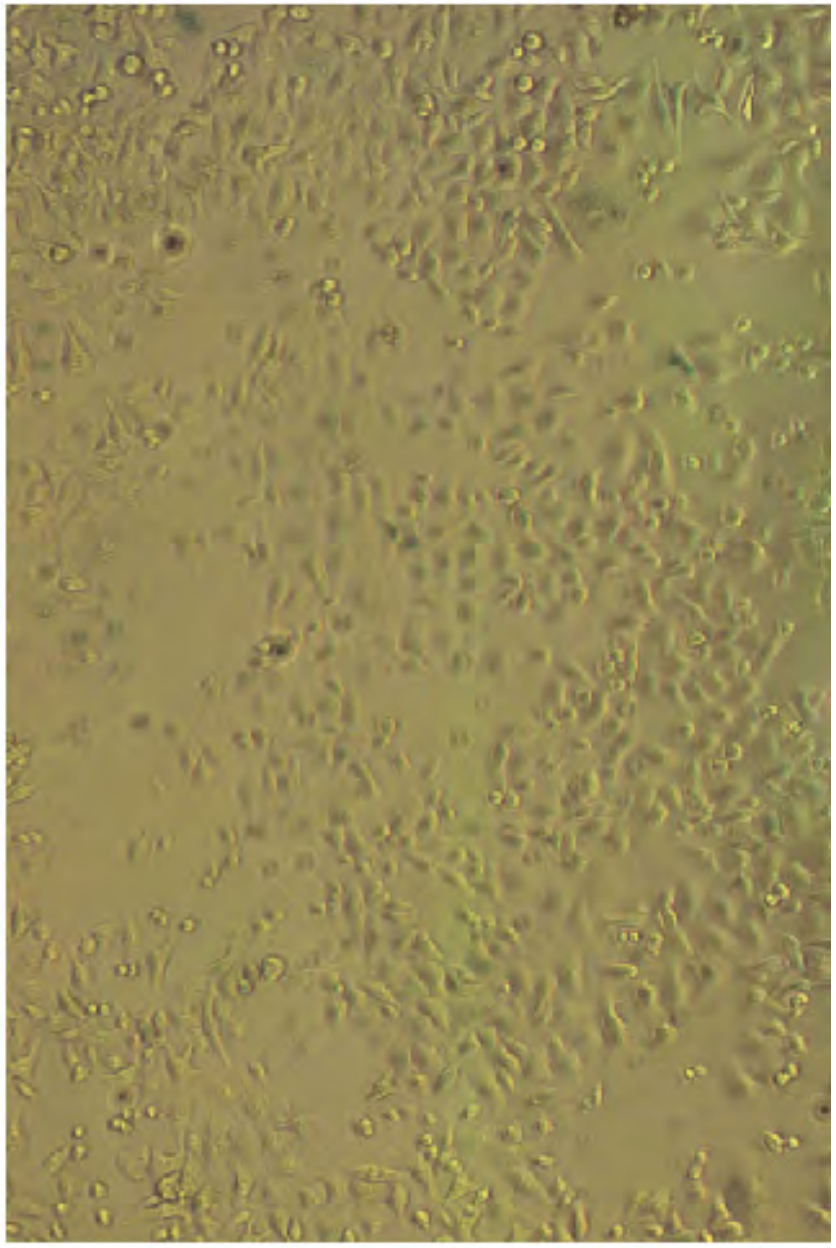
# Two patterns of conserved helical interactions



# C34 inhibits syncytia formation

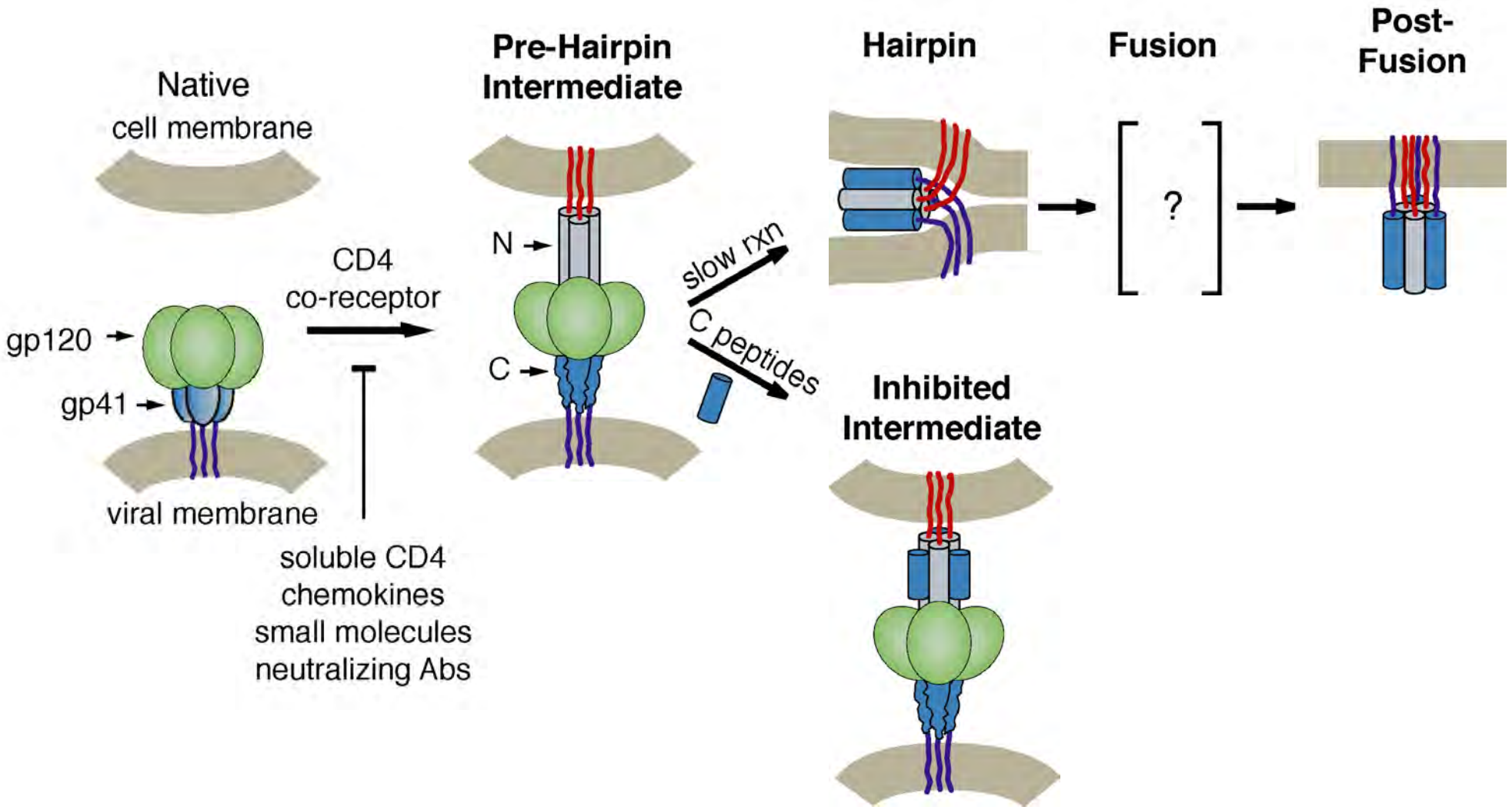


Control



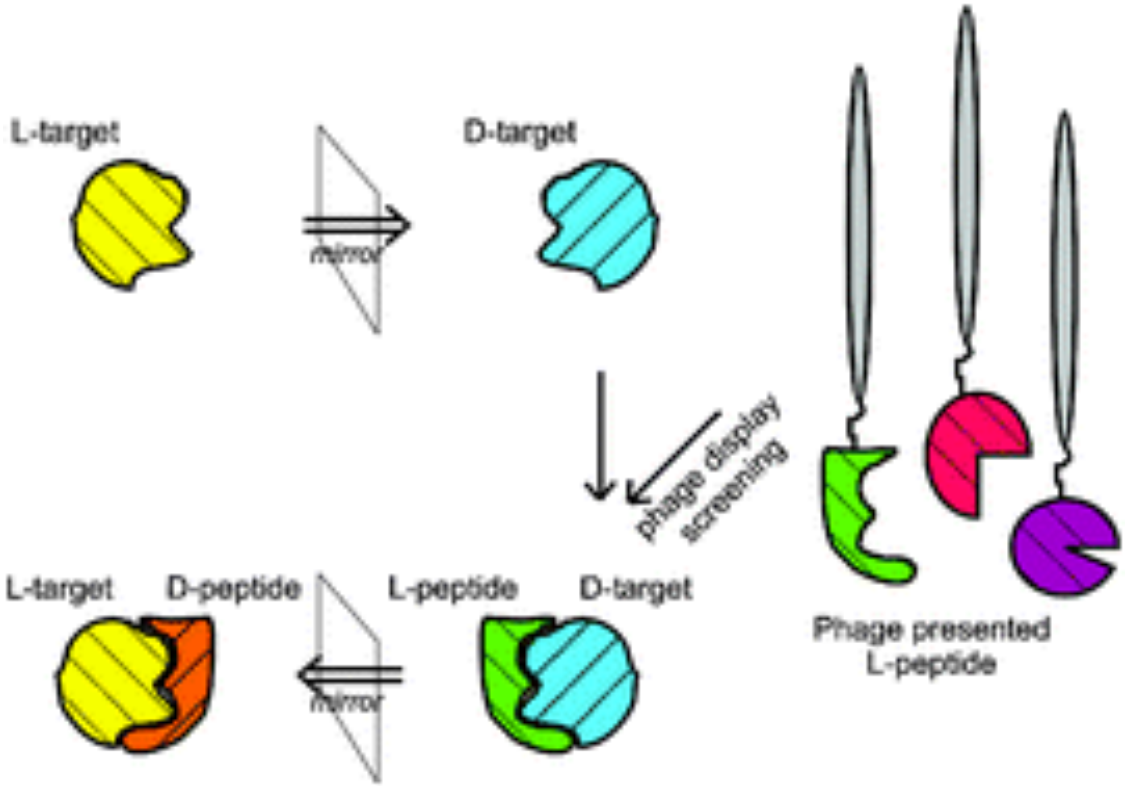
50 nM C34

# A model for HIV membrane fusion and its inhibition




Chan & Kim (1998) Cell 93: 681

# Mirror-image phage display to obtain D-peptide inhibitors

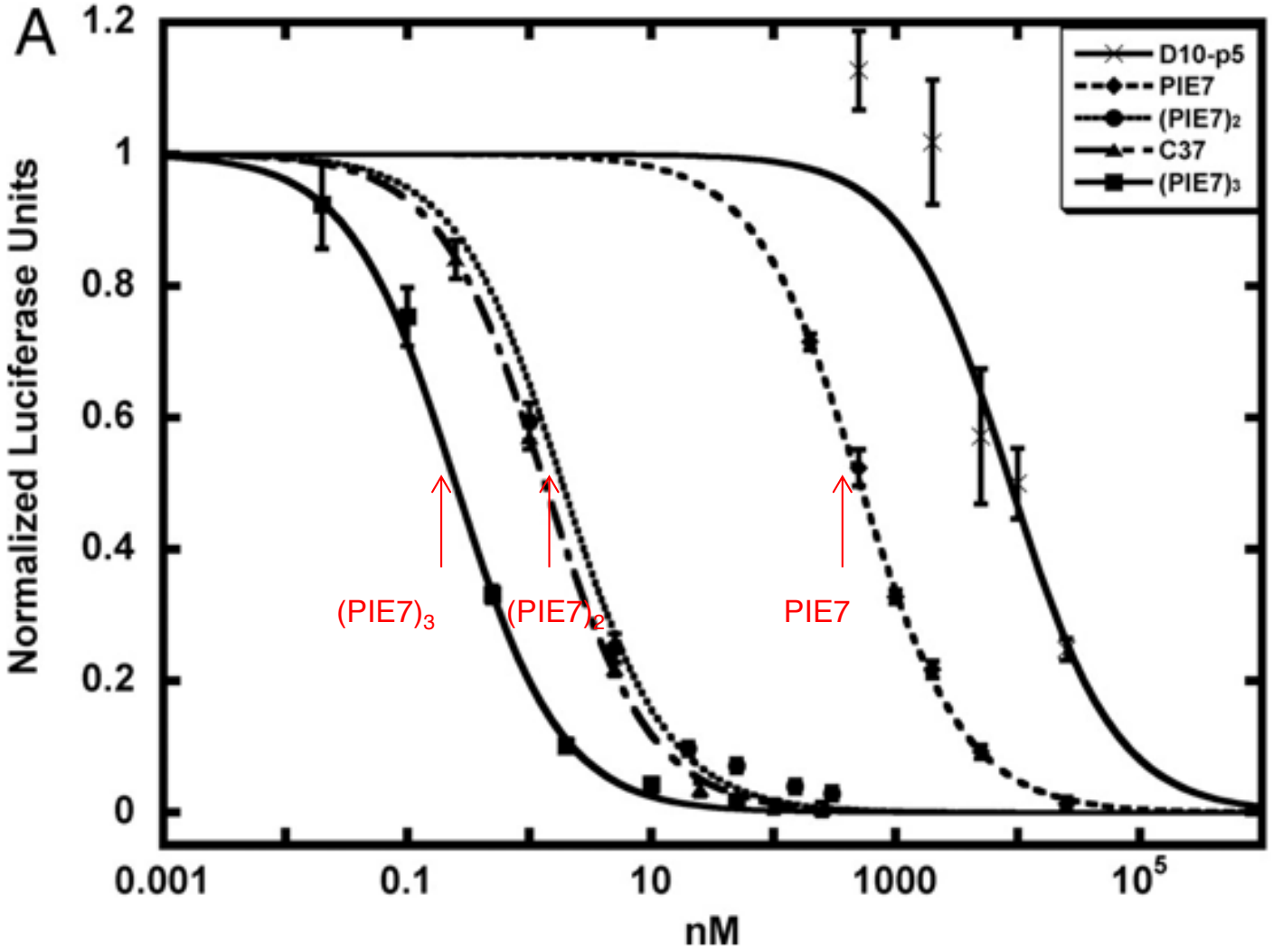



PIE7 is a D-peptide HIV-1 inhibitor with an $IC_{50}$ ~600 nM.

C37 is a C-peptide HIV-1 inhibitor with an $IC_{50}$~1.4 nM.

Welch et al (2007) PNAS

# Improvement of HIV-1 inhibitors via increases in avidity




PIE7 is a D-peptide HIV-1 inhibitor with an $IC_{50}$ ~600 nM.

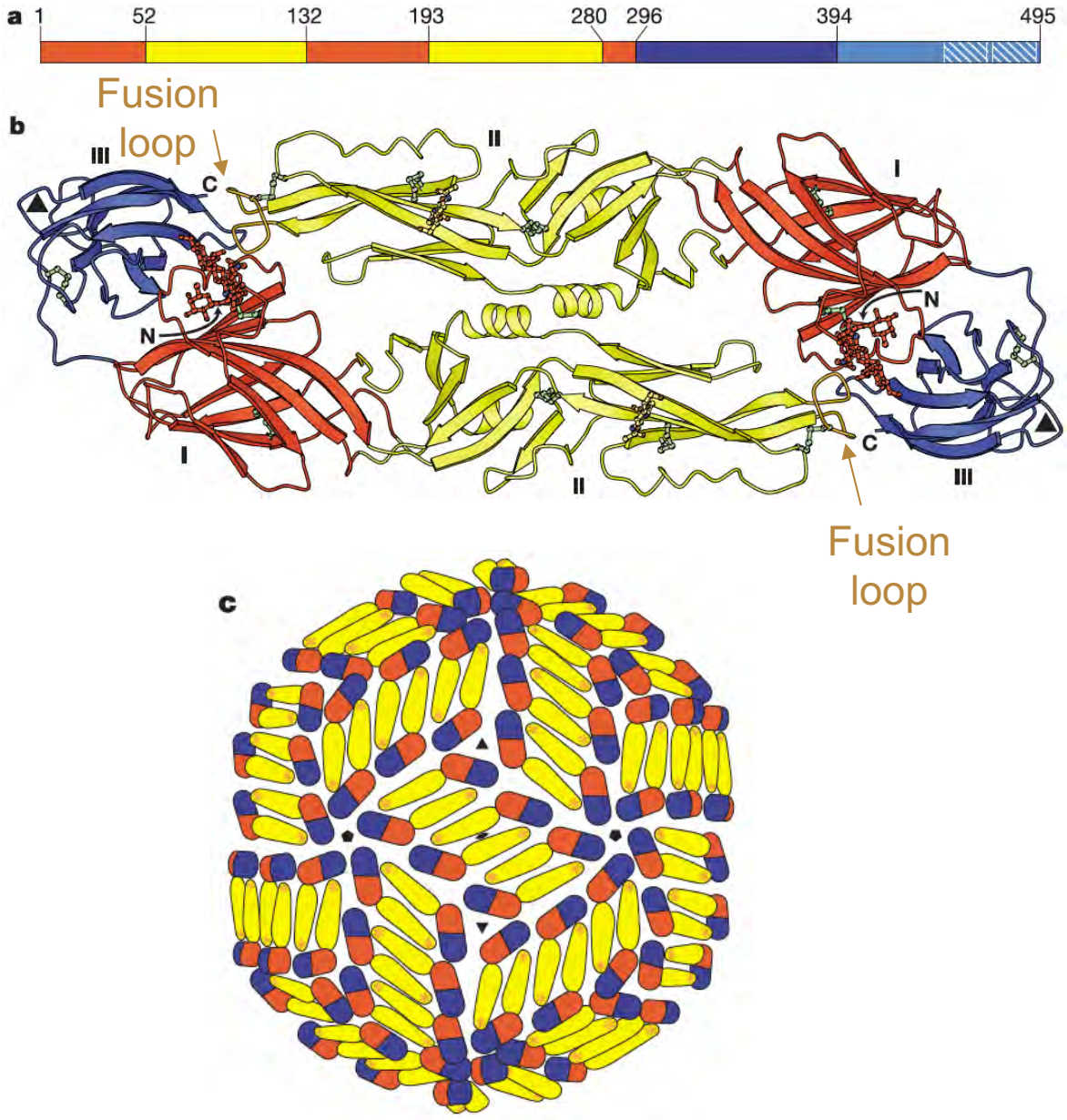
C37 is a C-peptide HIV-1 inhibitor with an $IC_{50}$~1.4 nM.

PIE7 was dimerized or trimerized with a bis(NHS ester)PEG crosslinker, which has a 35 angstrom PEG spacer.

$(PIE)_2$ $IC_{50}$~1.9 nM
$(PIE)_3$ $IC_{50}$~0.25 nM

Welch et al (2007) PNAS

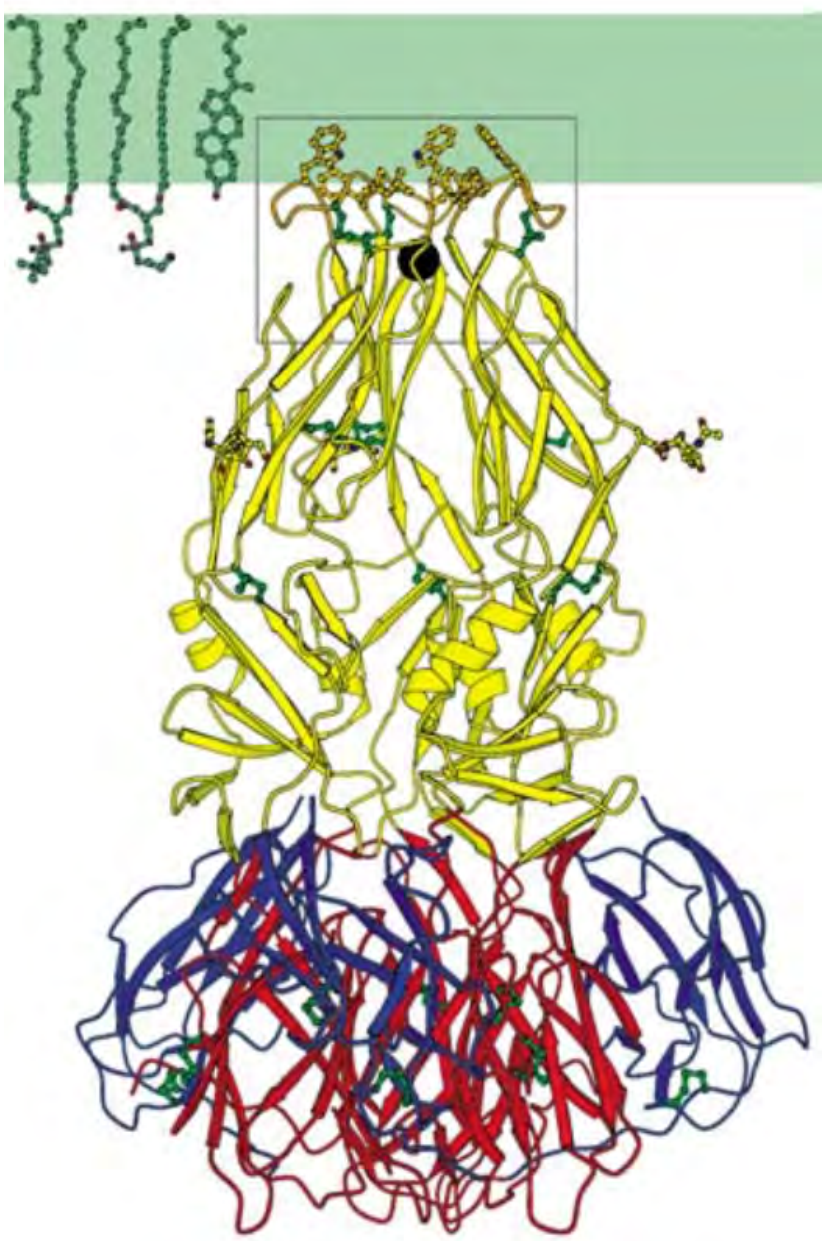
# Flaviviruses have fusogenic membrane proteins




- **Flavivirus**: enveloped, positive-sense, ssRNA
- West Nile, dengue, yellow fever, Zika, tick-borne encephalitis
- E is the “class II” fusion protein, activated by acidic pH.
- On the viral surface, E has 3 domains and is dimeric.
- Fusion loop is sequestered in hydrophobic interface.
- Fusogenic E is trimeric.

Modis et al (2004) Nature

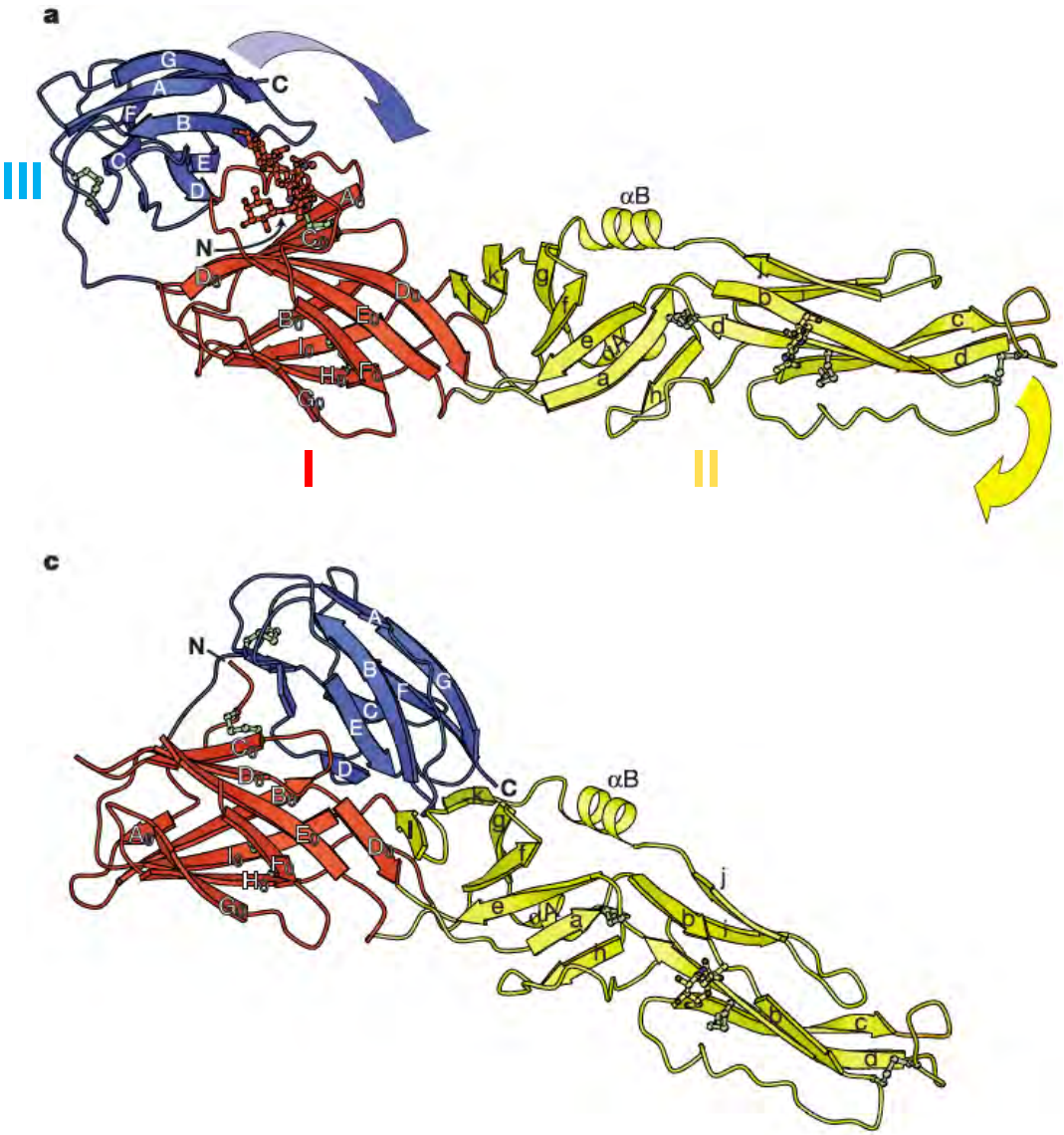
# The post-fusion trimeric structure



- The trimer uses a “aromatic anchor” of Trp and Phe to interact with target membrane.
- It is estimated that the fusion loop can only penetrate 6 angstroms into the outer leaflet, due to polar and charged residues beyond that.

Modis et al (2004) Nature

# Structural rearrangements in dimeric vs trimeric states

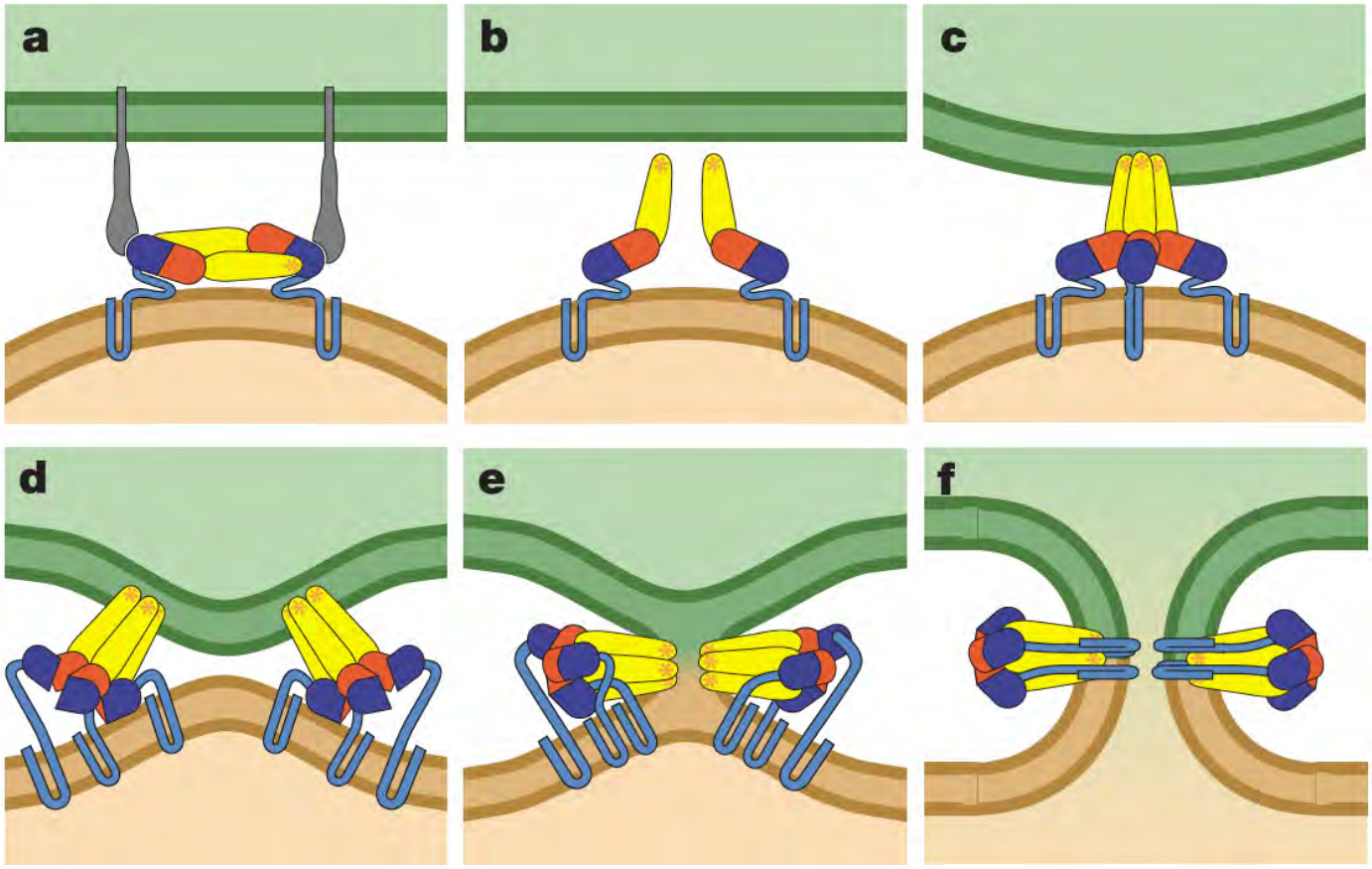



At the low pH of the endosome:

- E dimer dissociates into monomers that will become trimers.
- Fusion loop is exposed and inserts into target membrane.
- E trimer forms.
- Domain folds are similar; but domain II rotates 30˚; domain III rotates 70˚.
- The net result is that the viral membrane is pulled towards cellular membrane, by ~40 angstroms.

Modis et al (2004) Nature

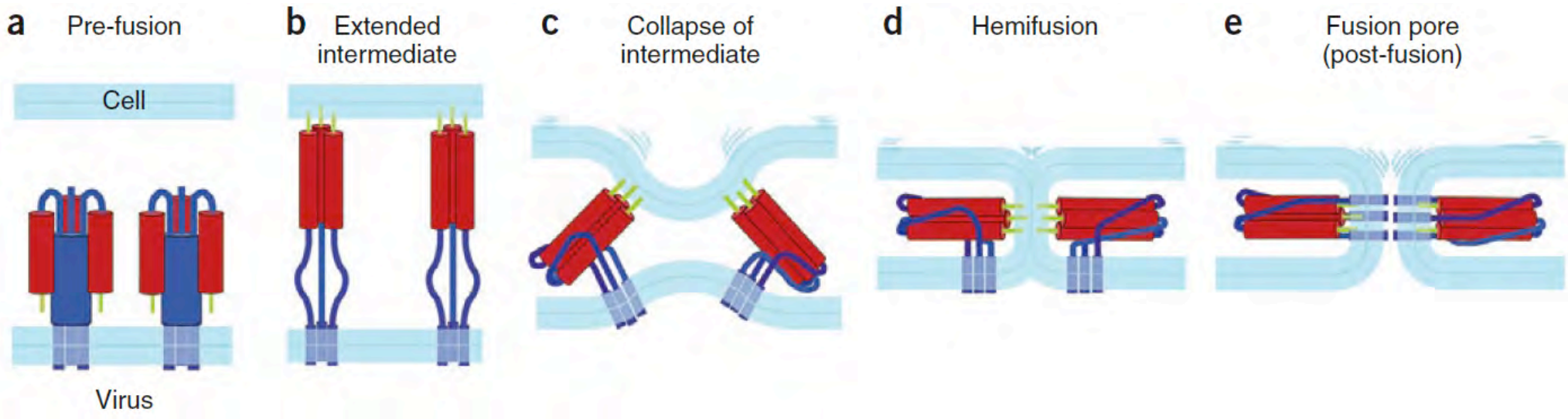
# Model for Dengue fusion




- In **b-c**, the low pH in endosome causes dimer to monomer to trimer transition.
- **c**, fusion loop interacts with cellular membrane.
- **d** and **e**, rearrangements bring viral membrane closer to target membrane.
- **e,** hemifusion intermediate

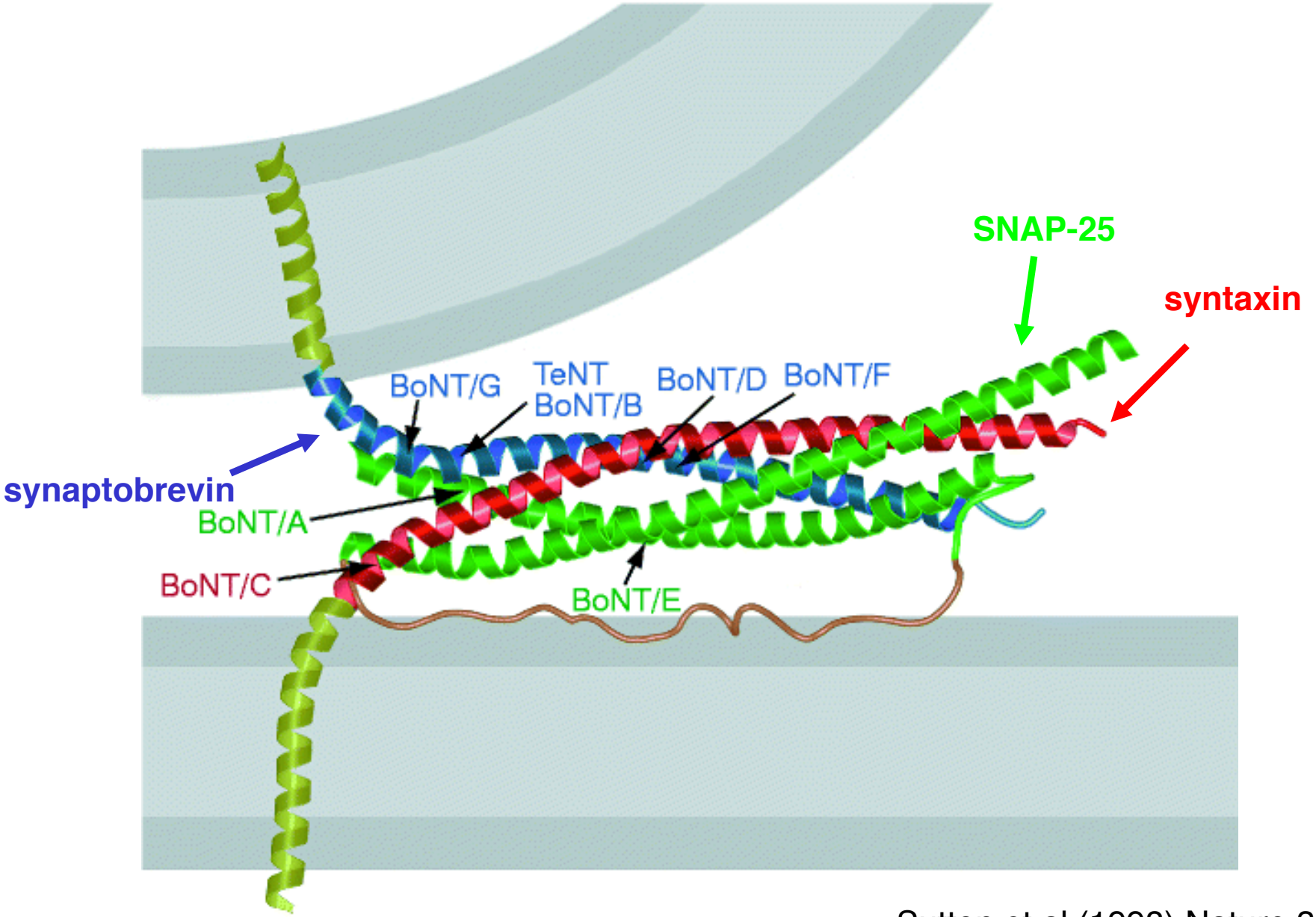
Modis et al (2004) Nature

# General features of viral fusion



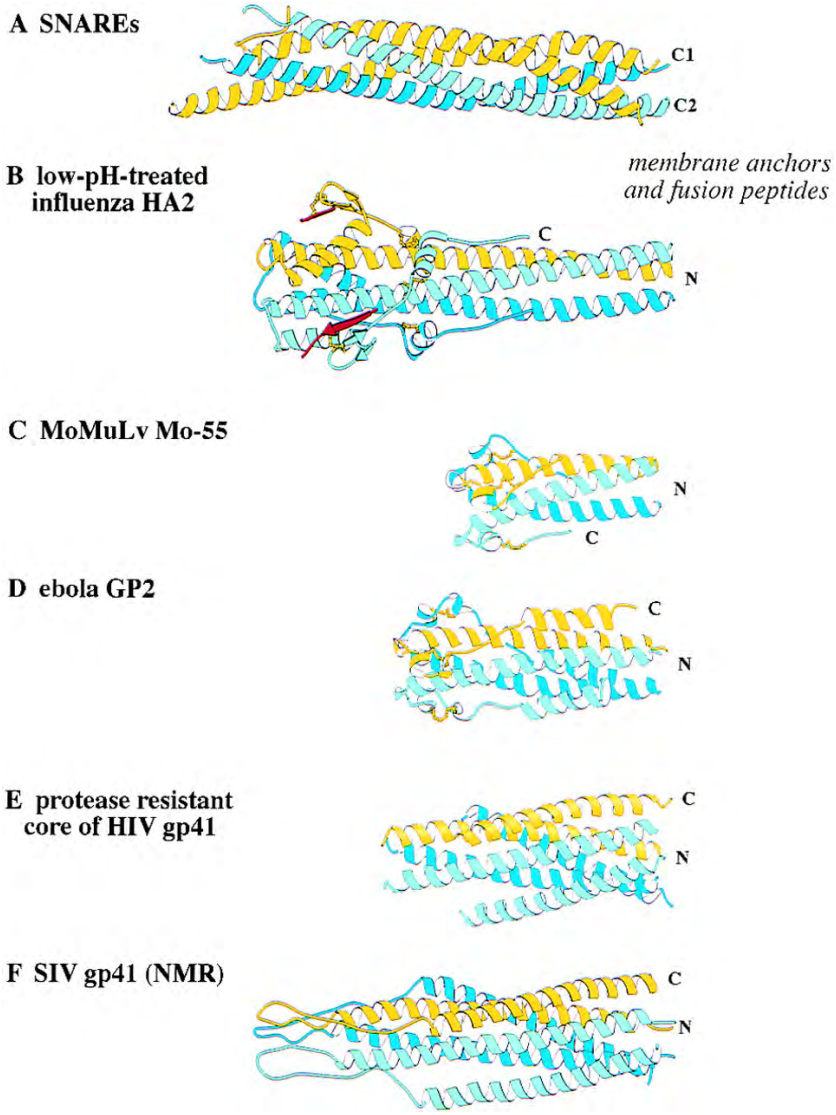

Harrison (2008) Nat Struct Mol Biol

# Model of SNARE complex in membrane fusion



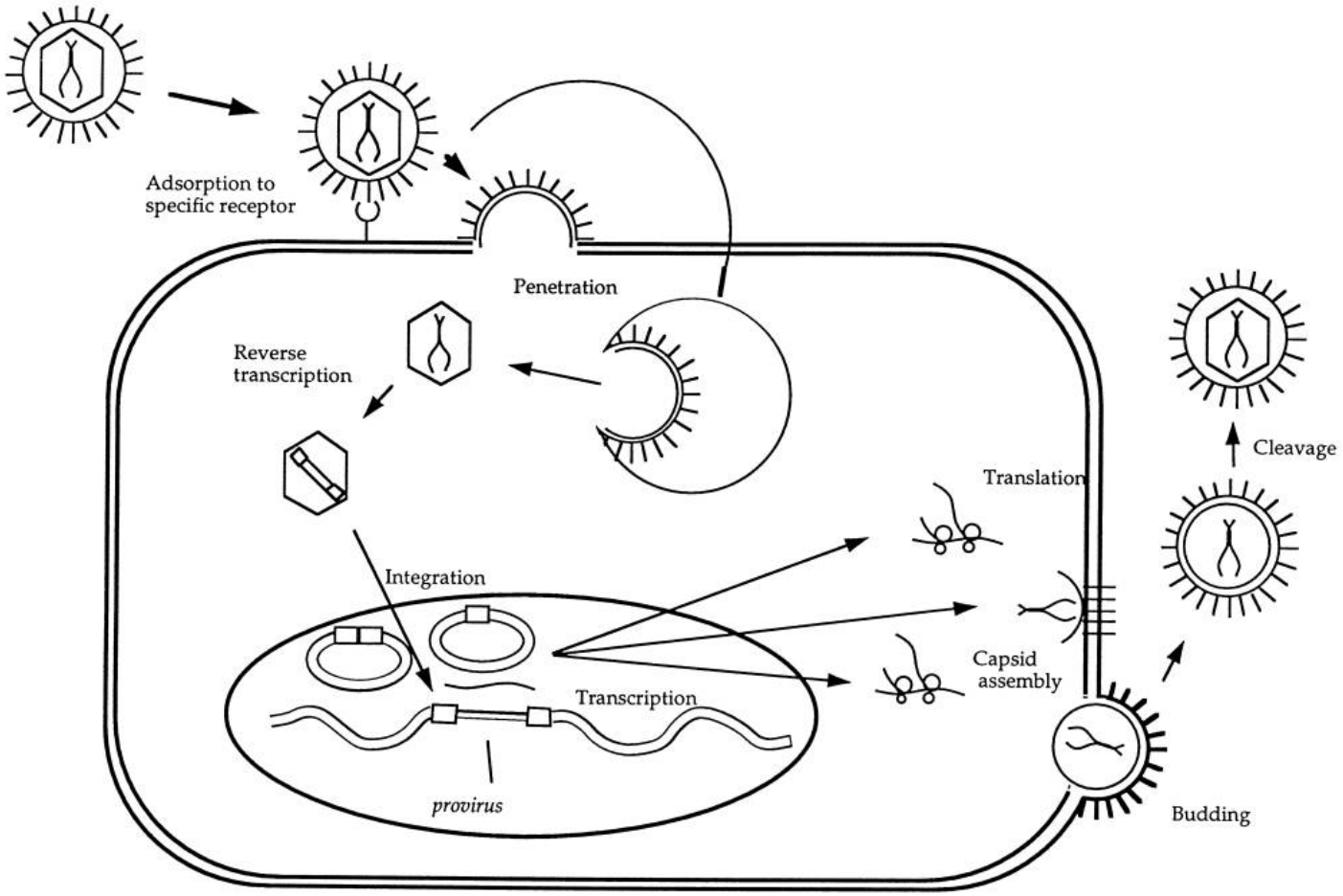

Sutton et al (1998) Nature 395:347

# Helical bundles involved in vesicle and viral membrane fusion




Skehel & Wiley (1998)
Cell

# Retroviral assembly and budding from the cell surface




- Retroviral budding is topologically opposite that of endocytosis
- Endocytosis buds into cytosol; virus particles bud away from cytosol
- Constriction of endocytic vesicles occurs by dynamin on the outside of the neck; viral buds constrict by proteins within the neck

Fields Virology, 2001