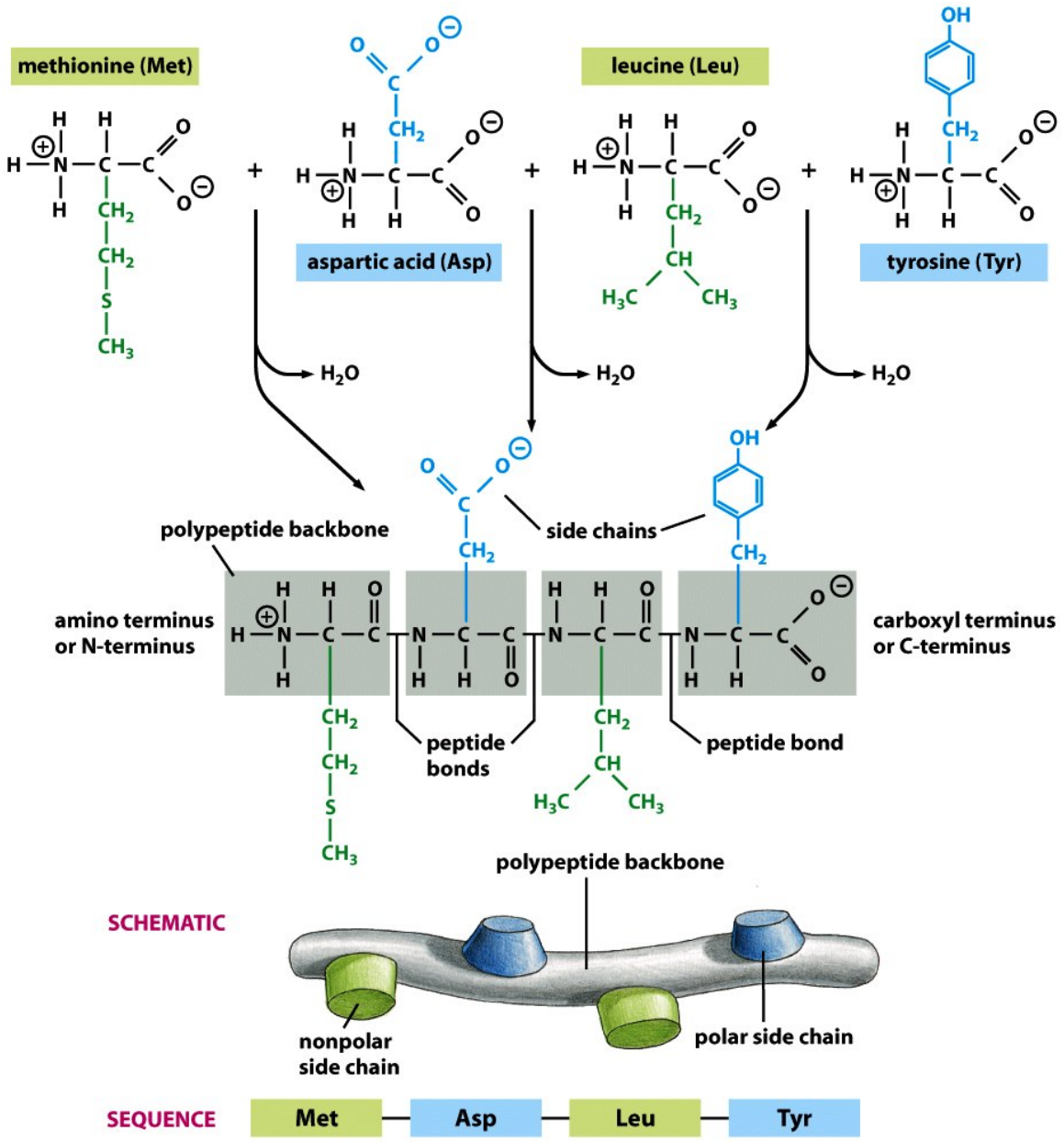
Proteins and Biomolecular Motors

- Introduction to proteins
- Introduction to biomolecular motors

References:

Bruce Alberts et al., Molecular Biology of the Cell (5th ed., 2007)

The components of a protein



Molecular Biology of the Cell (© Garland Science 2008)

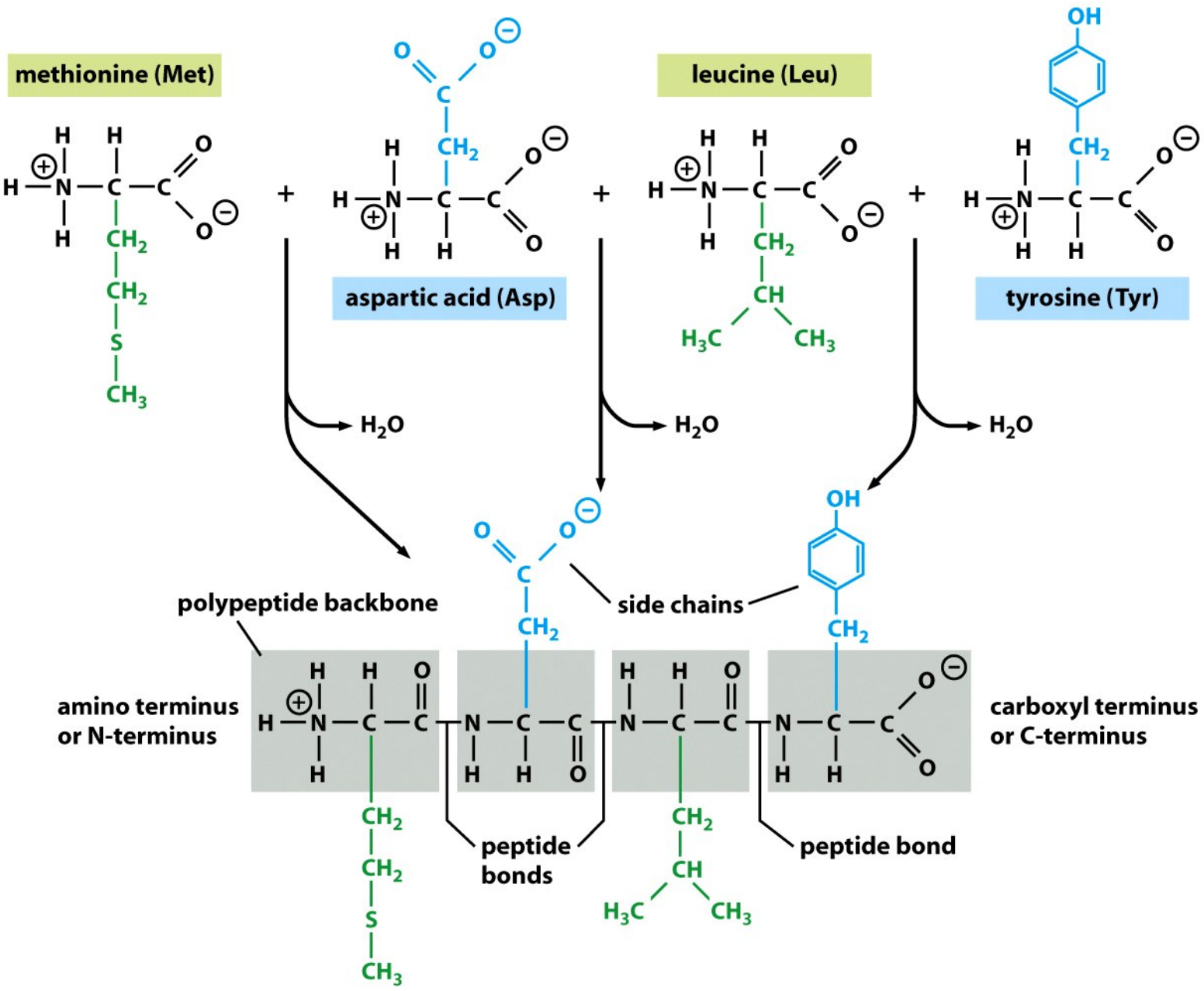


Figure 3-1 (part 1 of 2) *Molecular Biology of the Cell* (© Garland Science 2008)

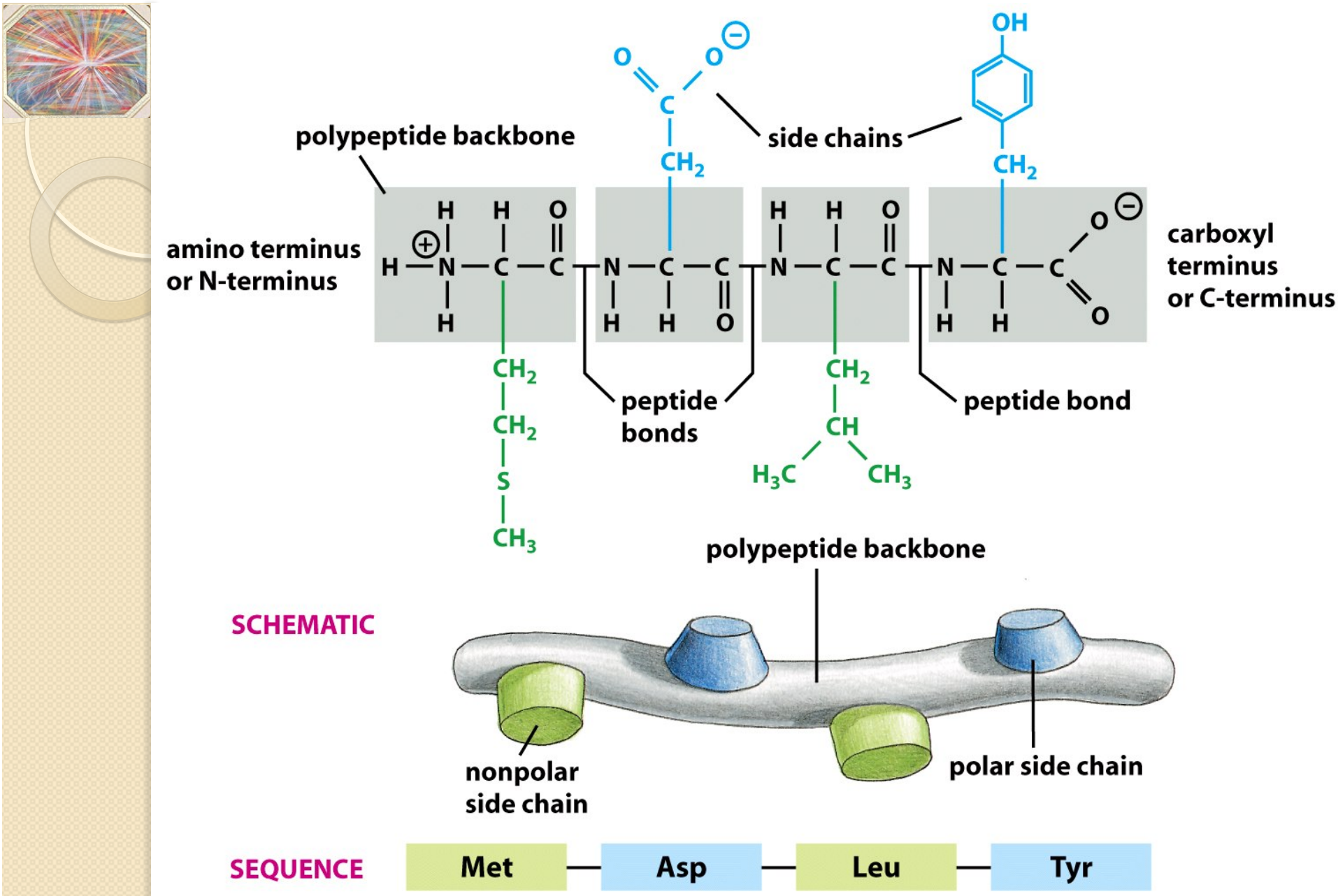


Figure 3-1 (part 2 of 2) ***Molecular Biology of the Cell*** **(© Garland Science 2008)**

The 20 amino acids found in proteins

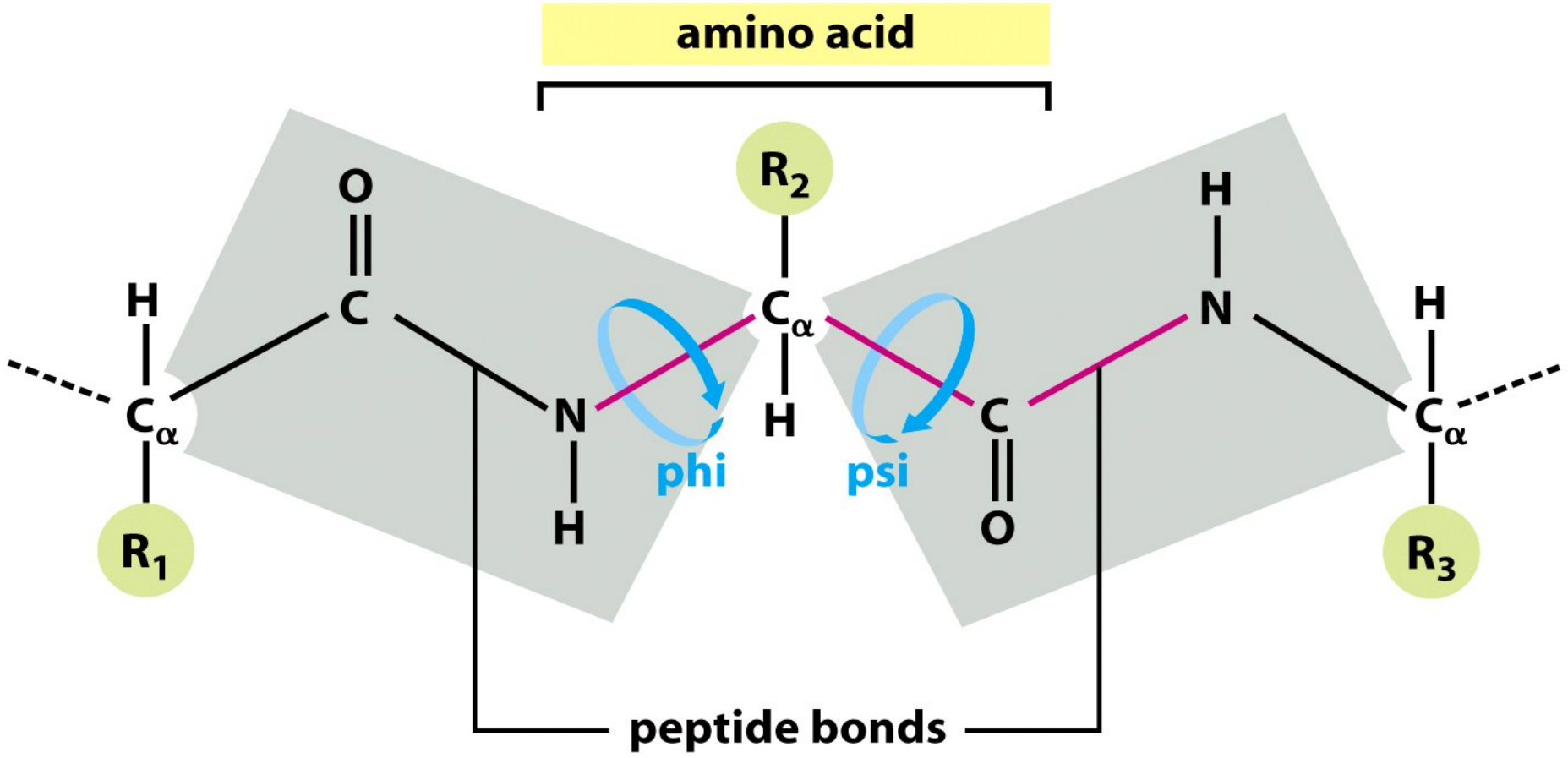
AMINO ACID			SIDE CHAIN
Aspartic acid	Asp	D	negative
Glutamic acid	Glu	E	negative
Arginine	Arg	R	positive
Lysine	Lys	K	positive
Histidine	His	H	positive
Asparagine	Asn	N	uncharged polar
Glutamine	Gln	Q	uncharged polar
Serine	Ser	S	uncharged polar
Threonine	Thr	T	uncharged polar
Tyrosine	Tyr	Y	uncharged polar

POLAR AMINO ACIDS

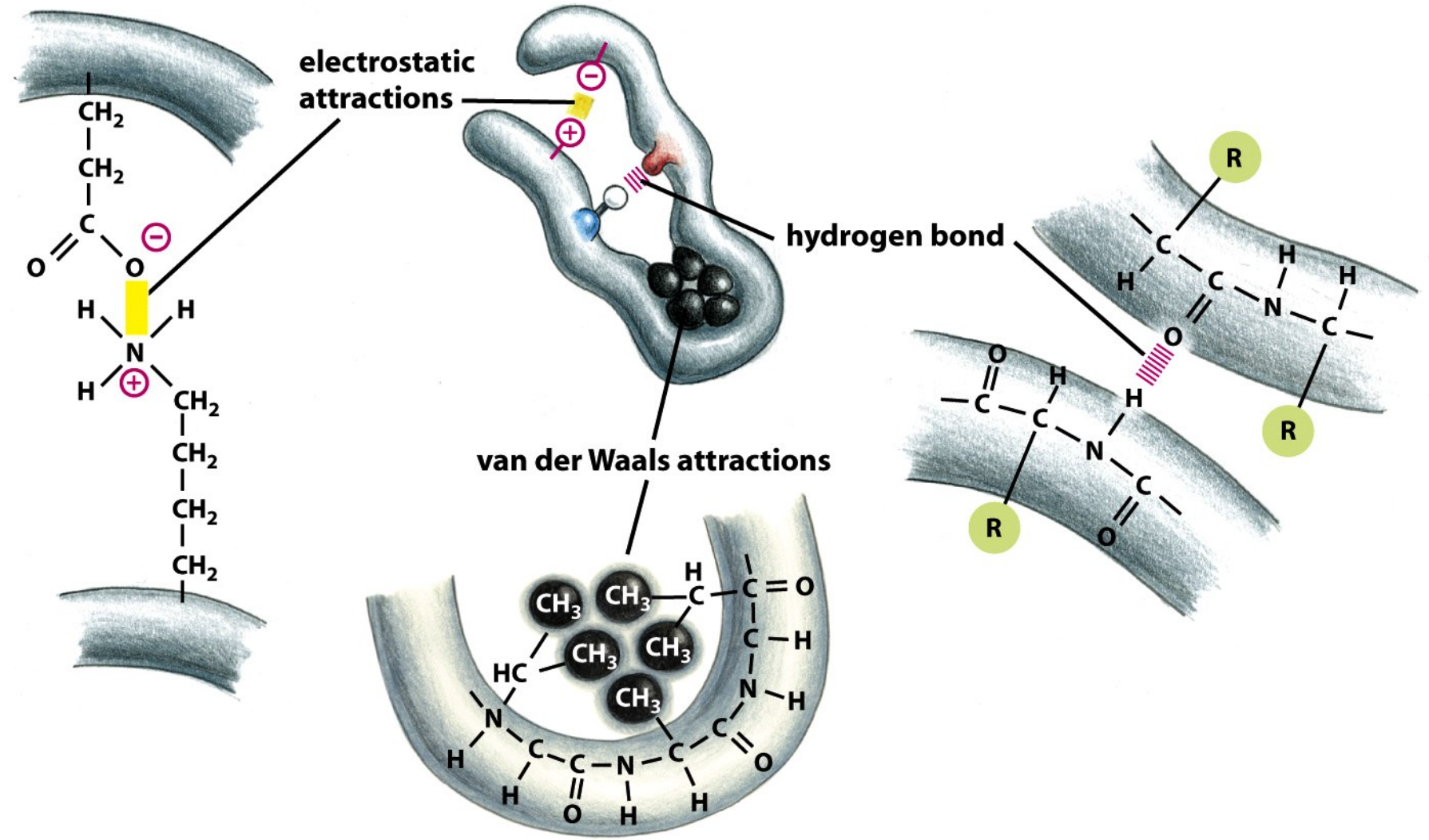
AMINO ACID			SIDE CHAIN
Alanine	Ala	A	nonpolar
Glycine	Gly	G	nonpolar
Valine	Val	V	nonpolar
Leucine	Leu	L	nonpolar
Isoleucine	Ile	I	nonpolar
Proline	Pro	P	nonpolar
Phenylalanine	Phe	F	nonpolar
Methionine	Met	M	nonpolar
Tryptophan	Trp	W	nonpolar
Cysteine	Cys	C	nonpolar

NONPOLAR AMINO ACIDS

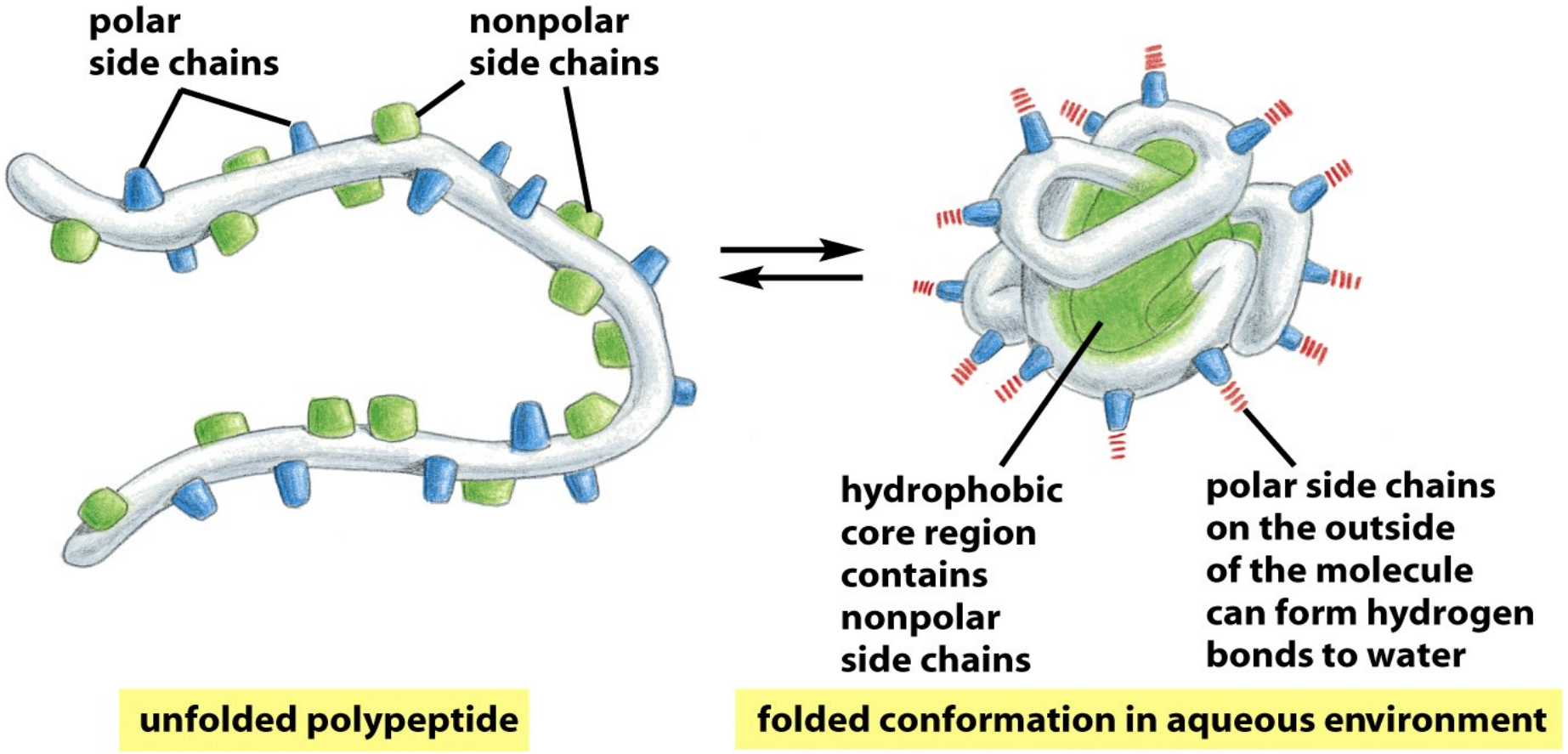
The steric limitations on the bond angles in a polypeptide chain



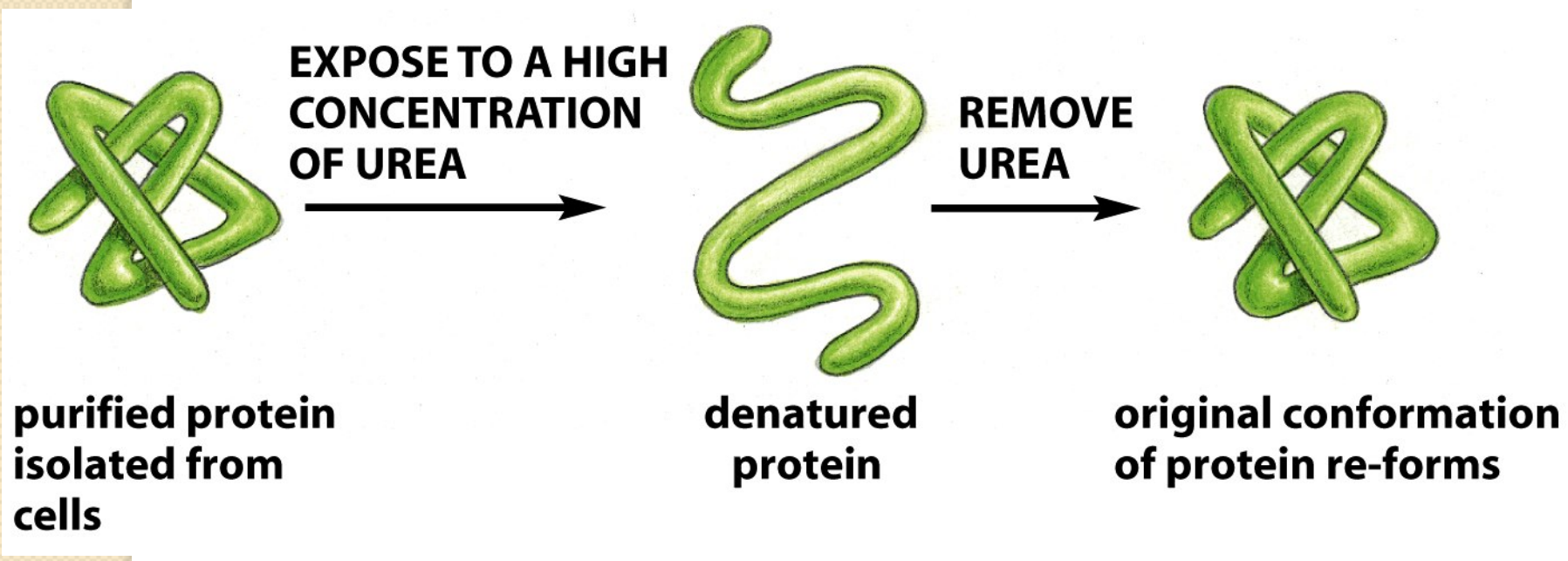
Three types of noncovalent bonds help proteins fold



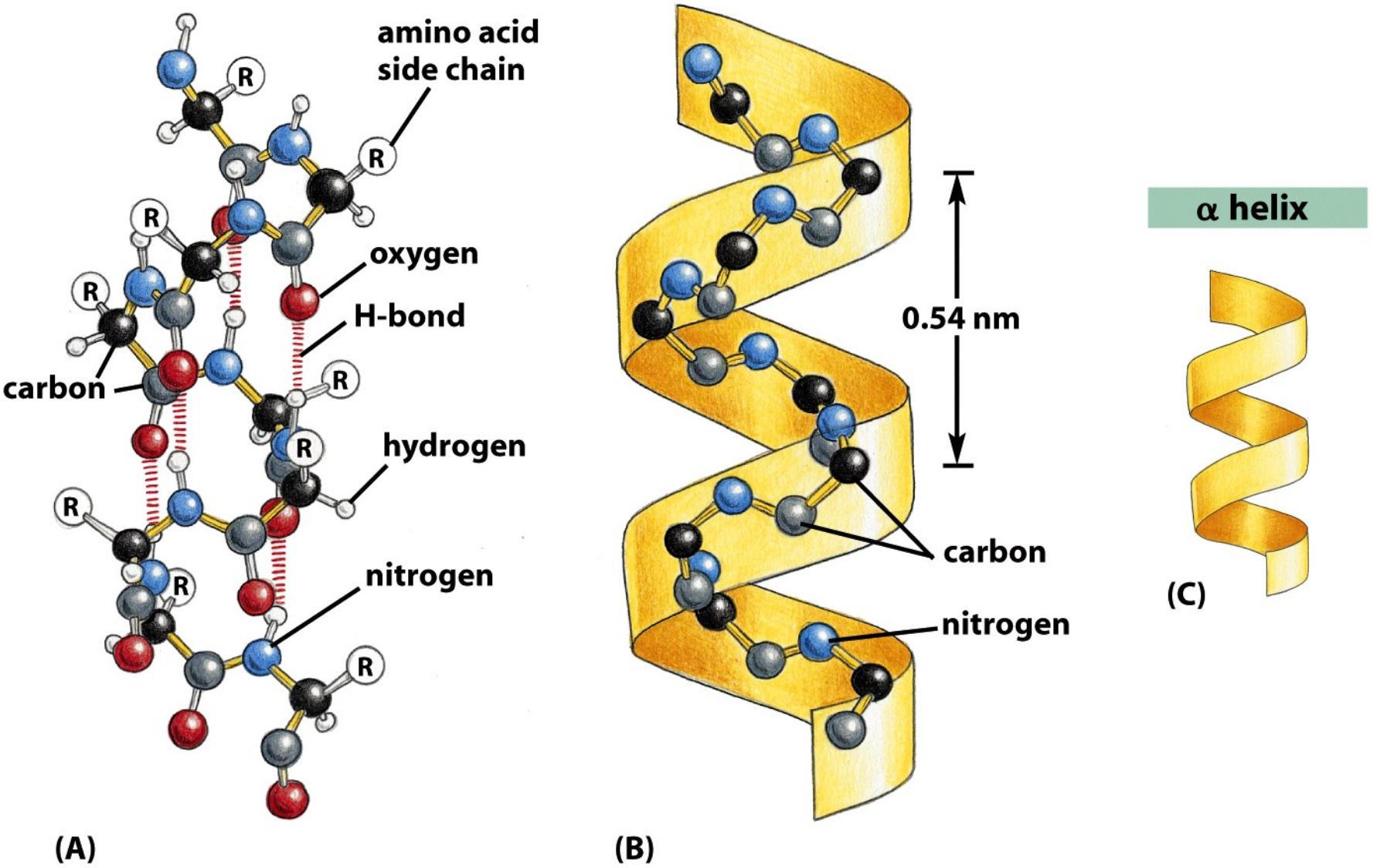
How a protein folds into a compact conformation



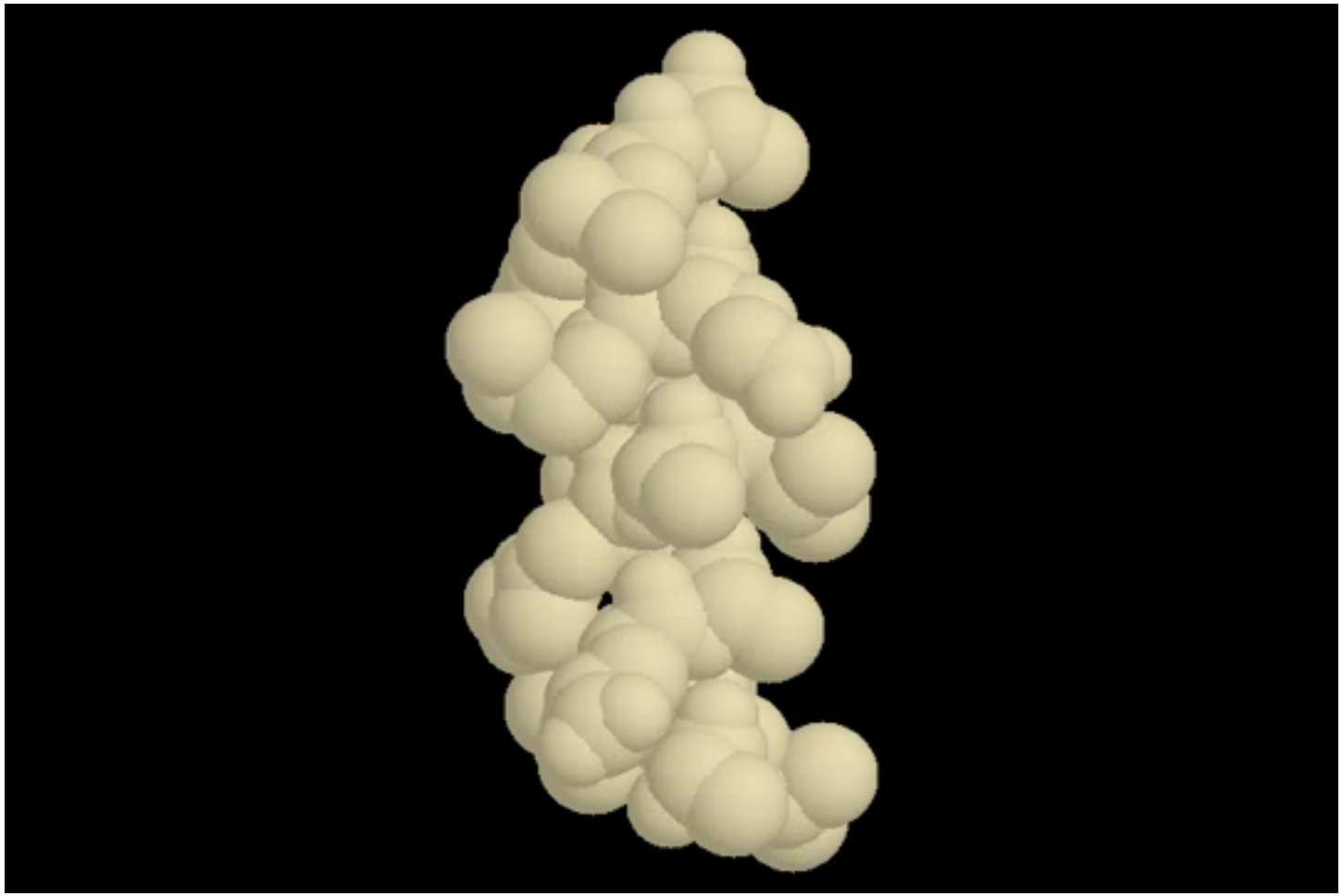
The folding of a denatured protein



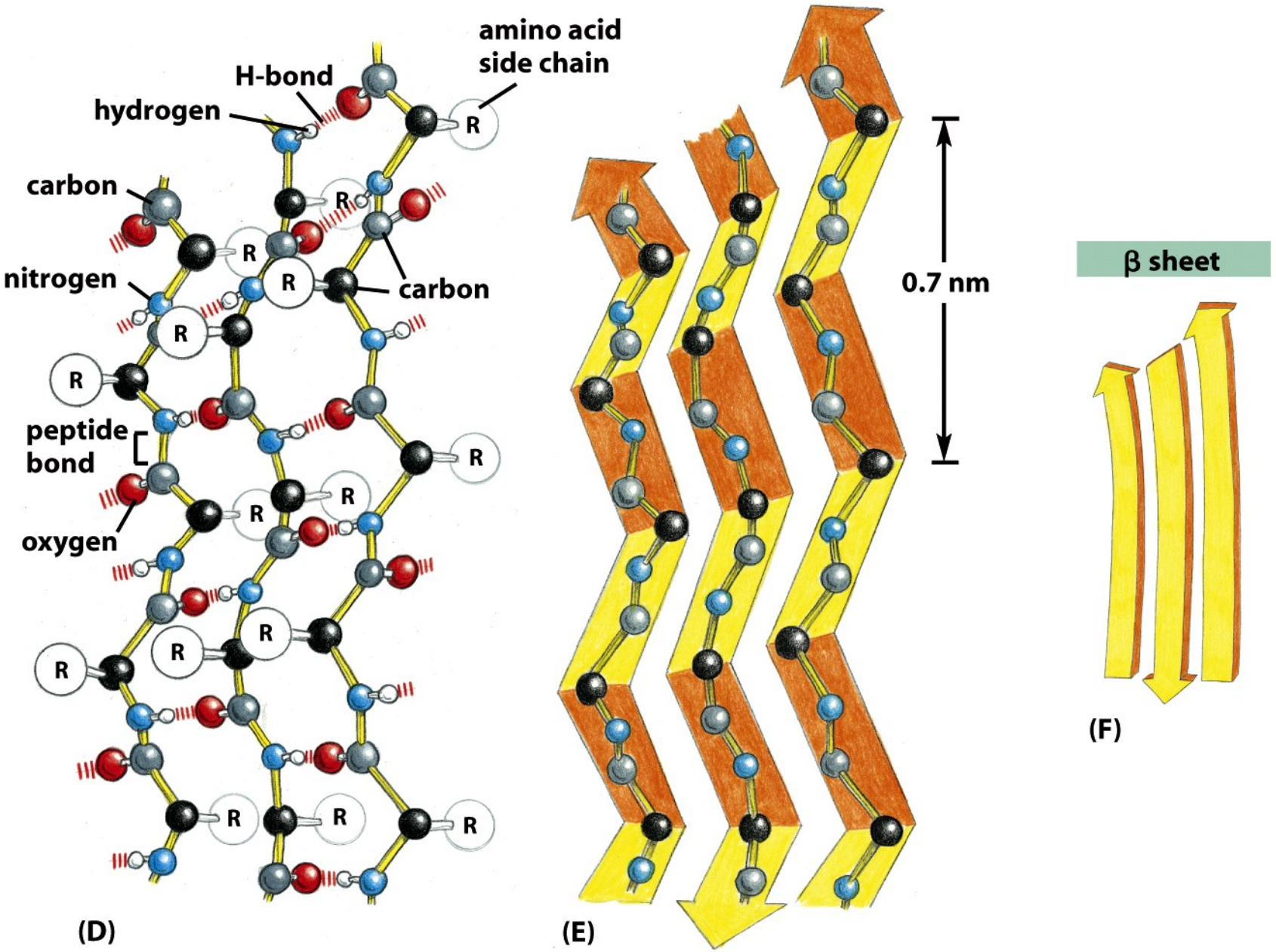
α helix



α helix (movie)



β sheet



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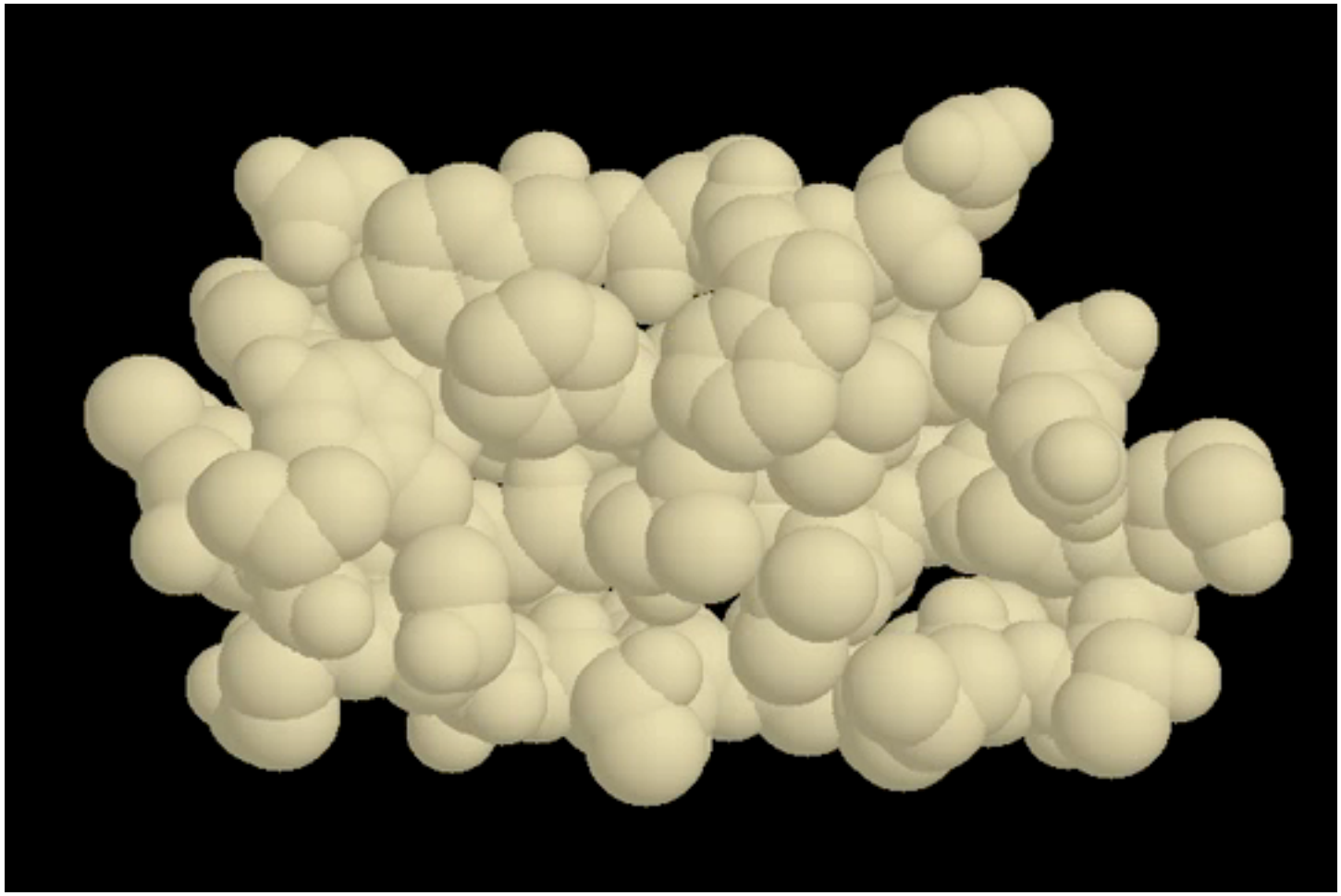
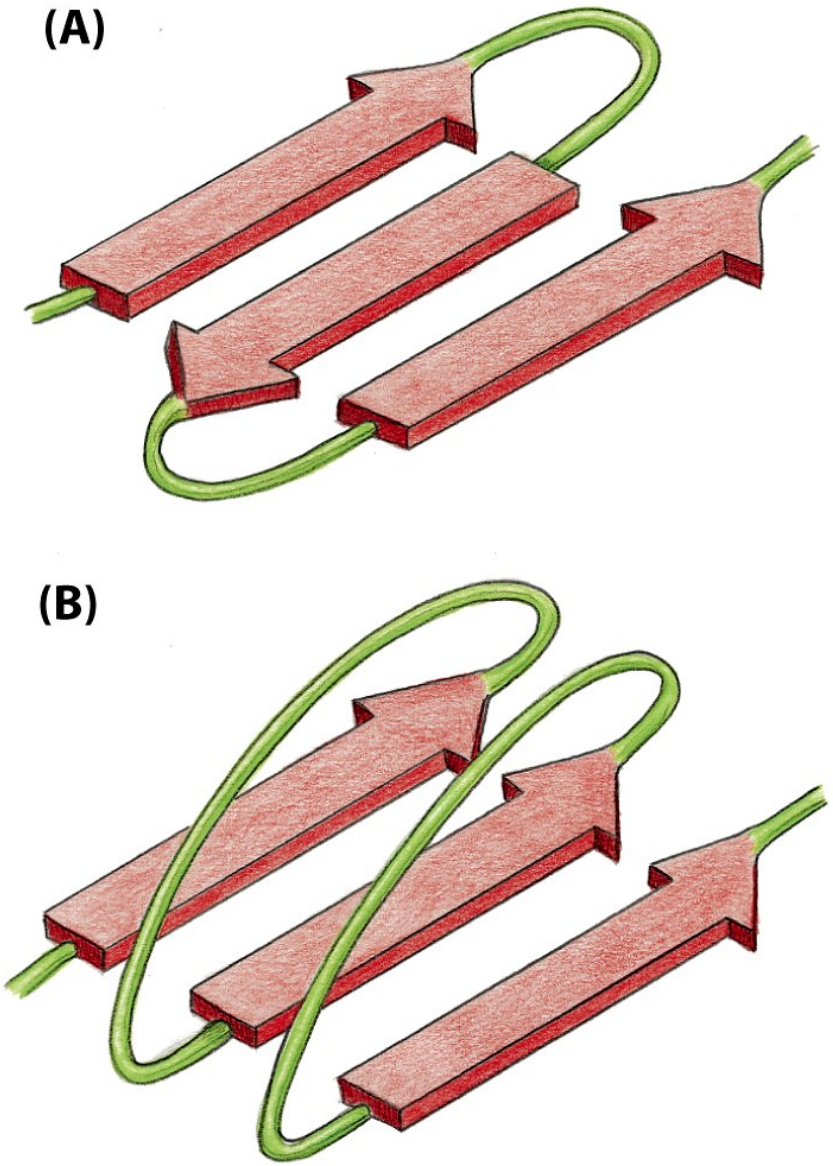
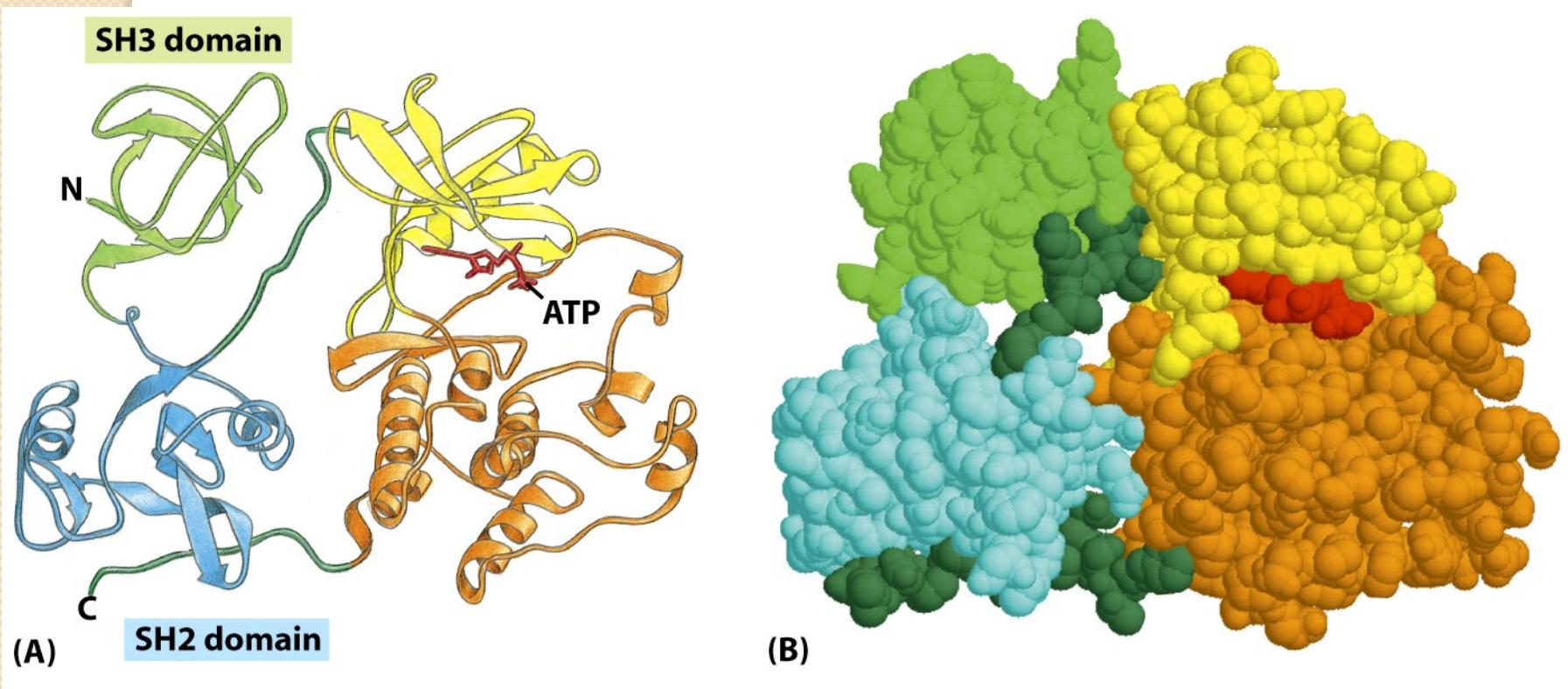
β sheet (movie)



Two types of β sheet structure



A protein with multiple domains



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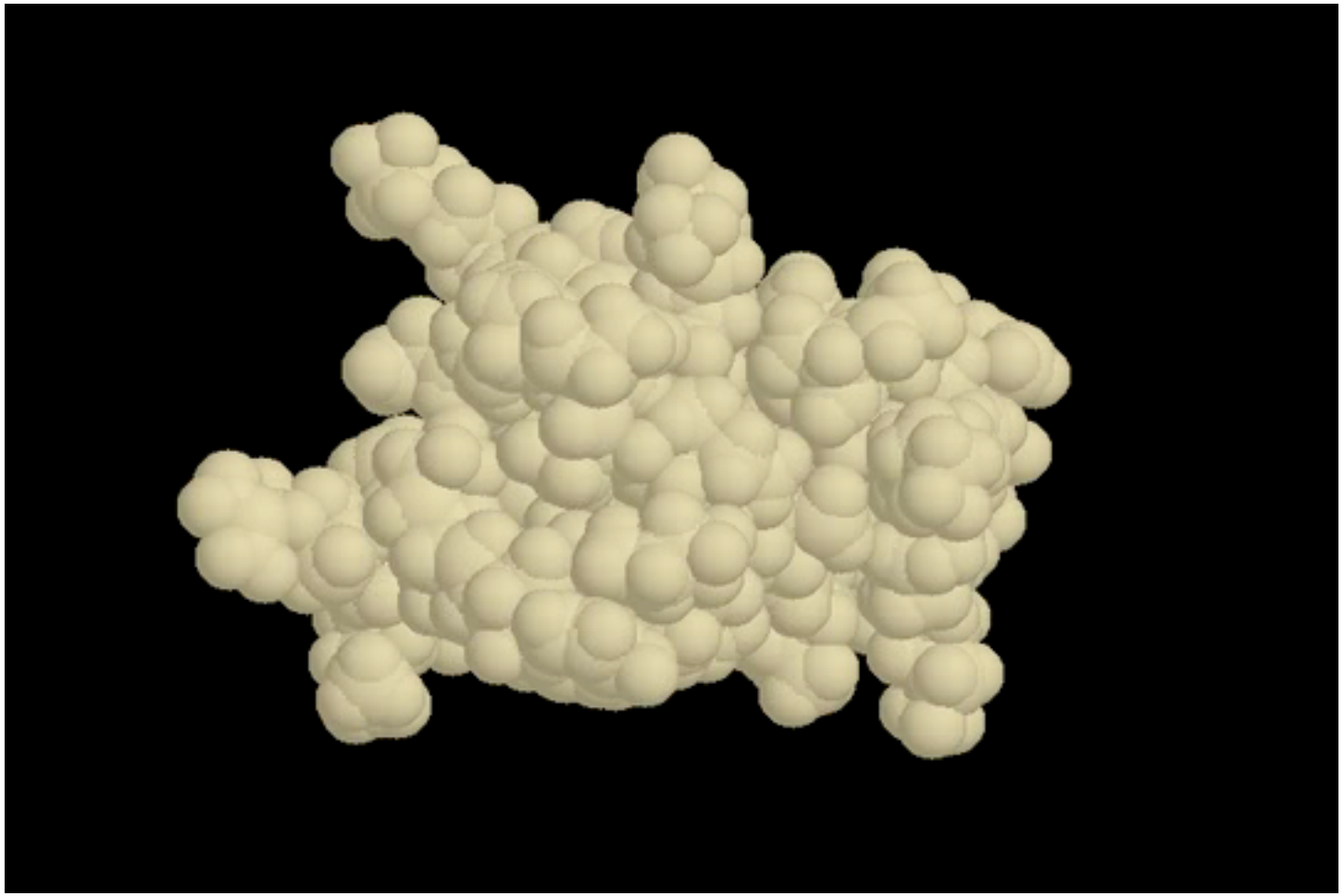
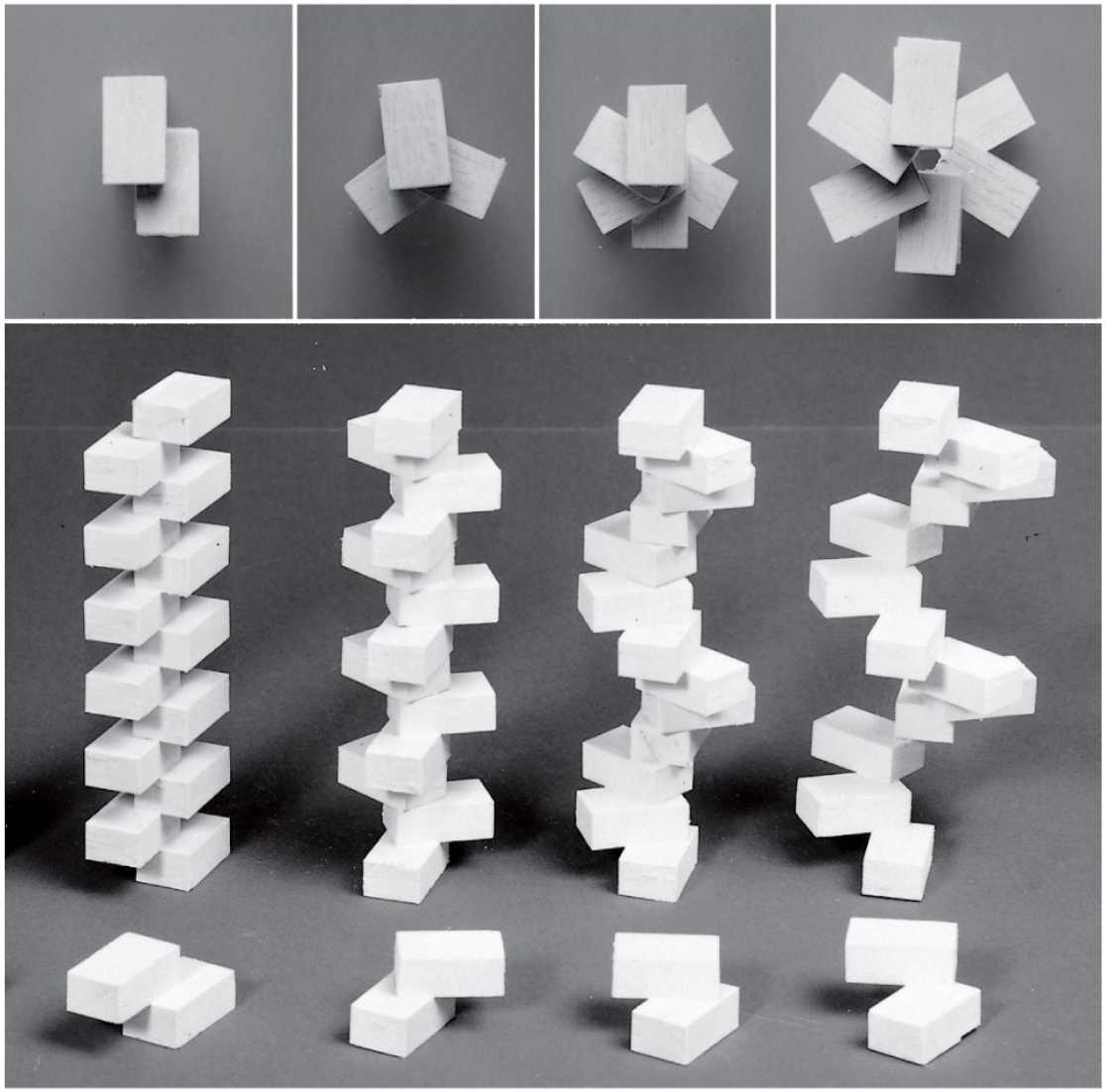
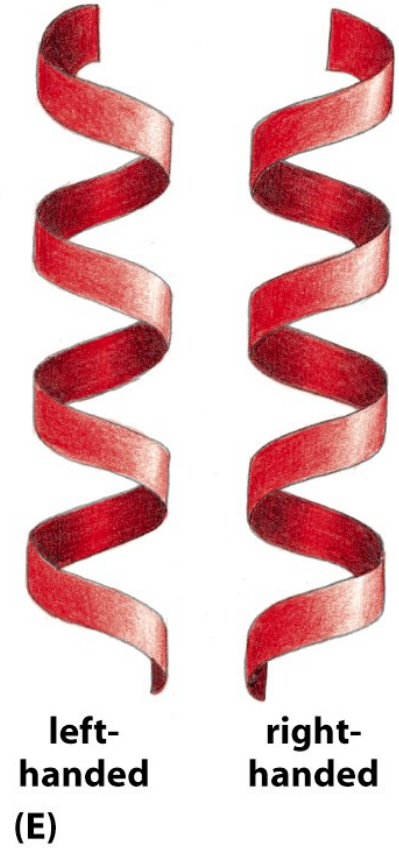
Disulfide bond (movie)

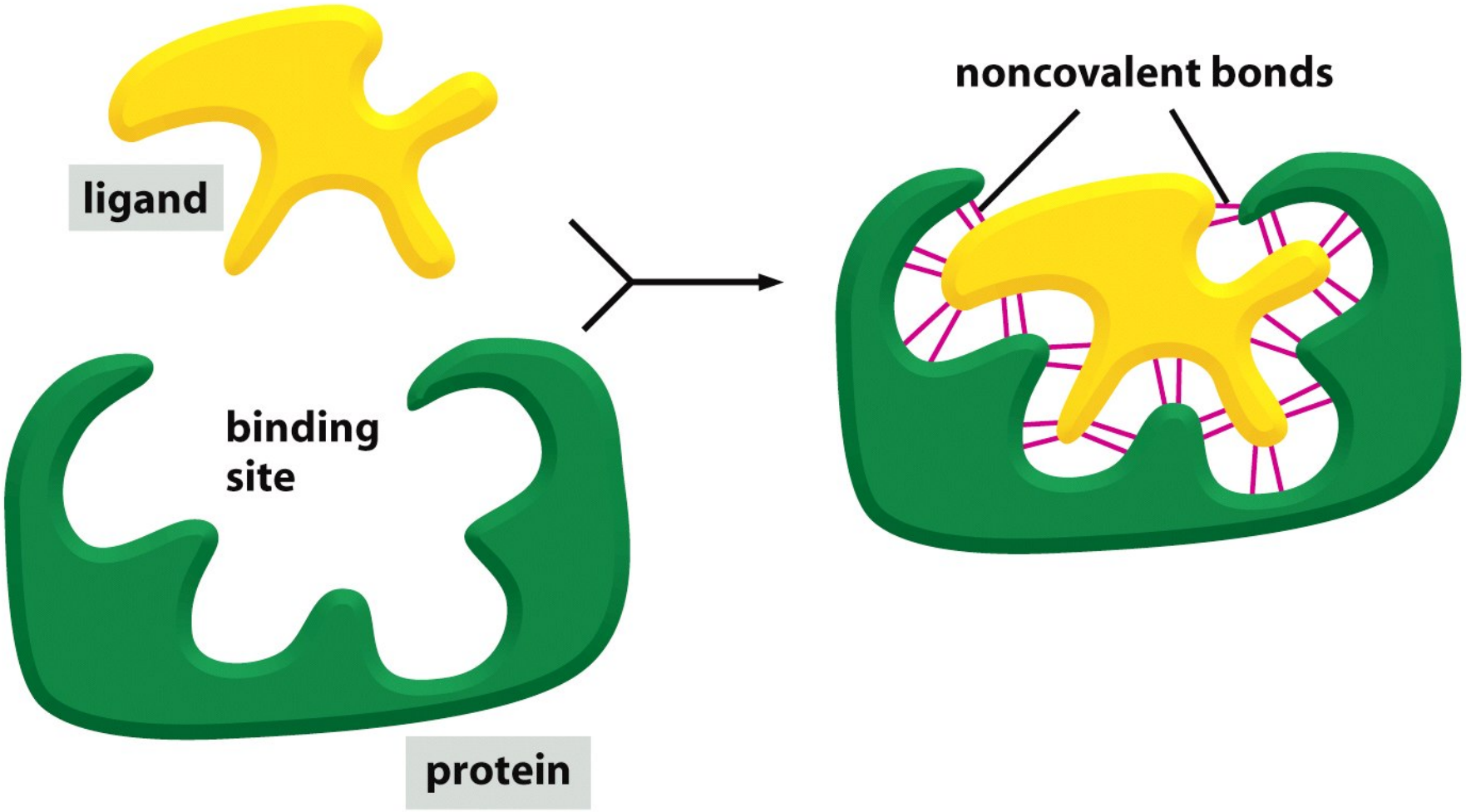


Some properties of a helix





The selective binding of a protein



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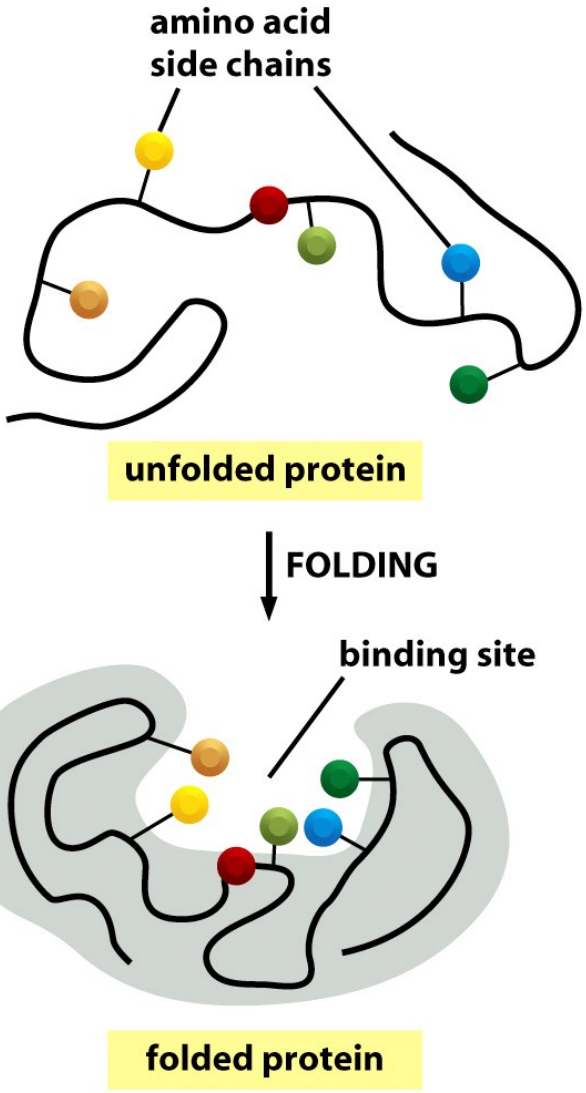
The binding site of a protein



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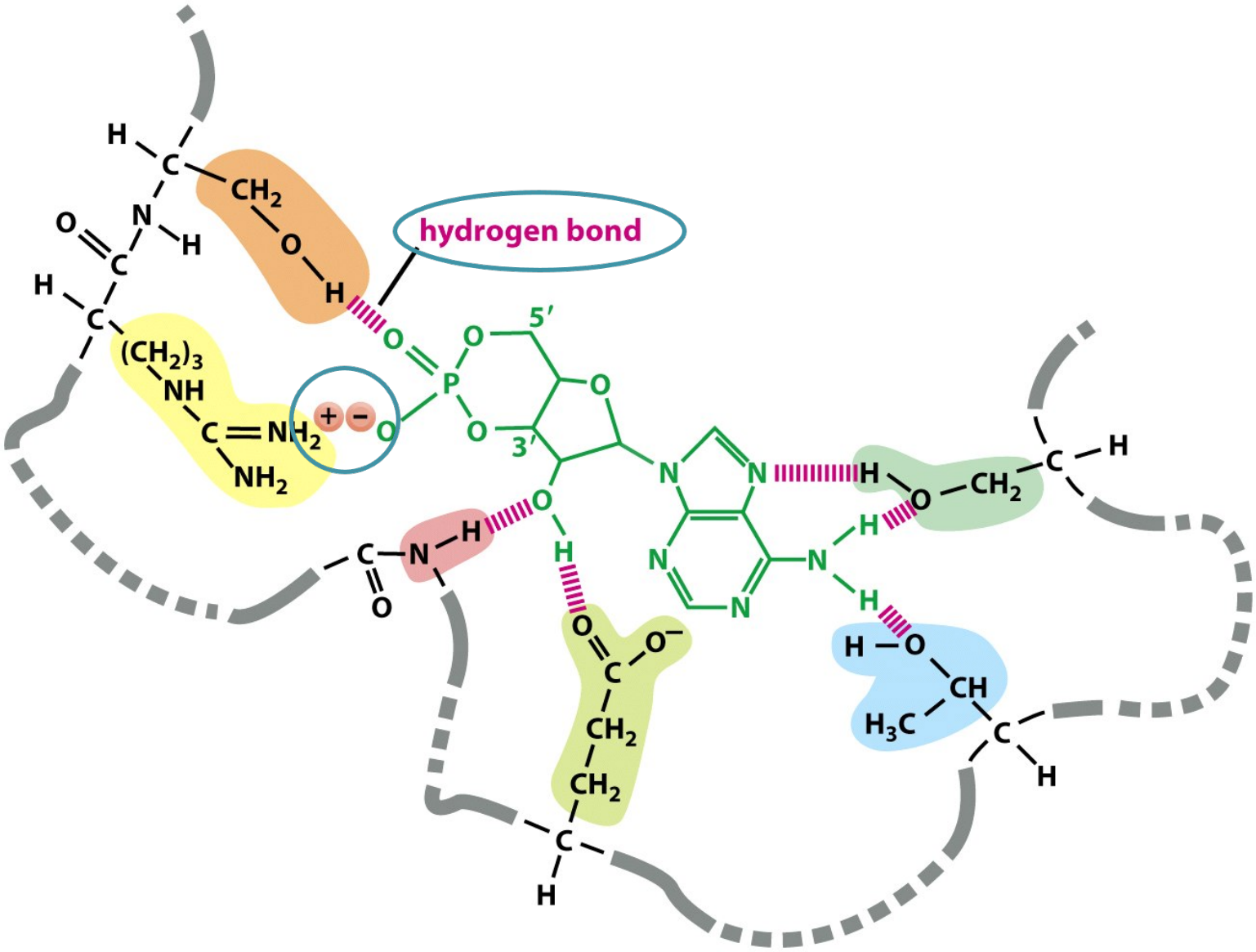
The binding site of a protein (close-up)



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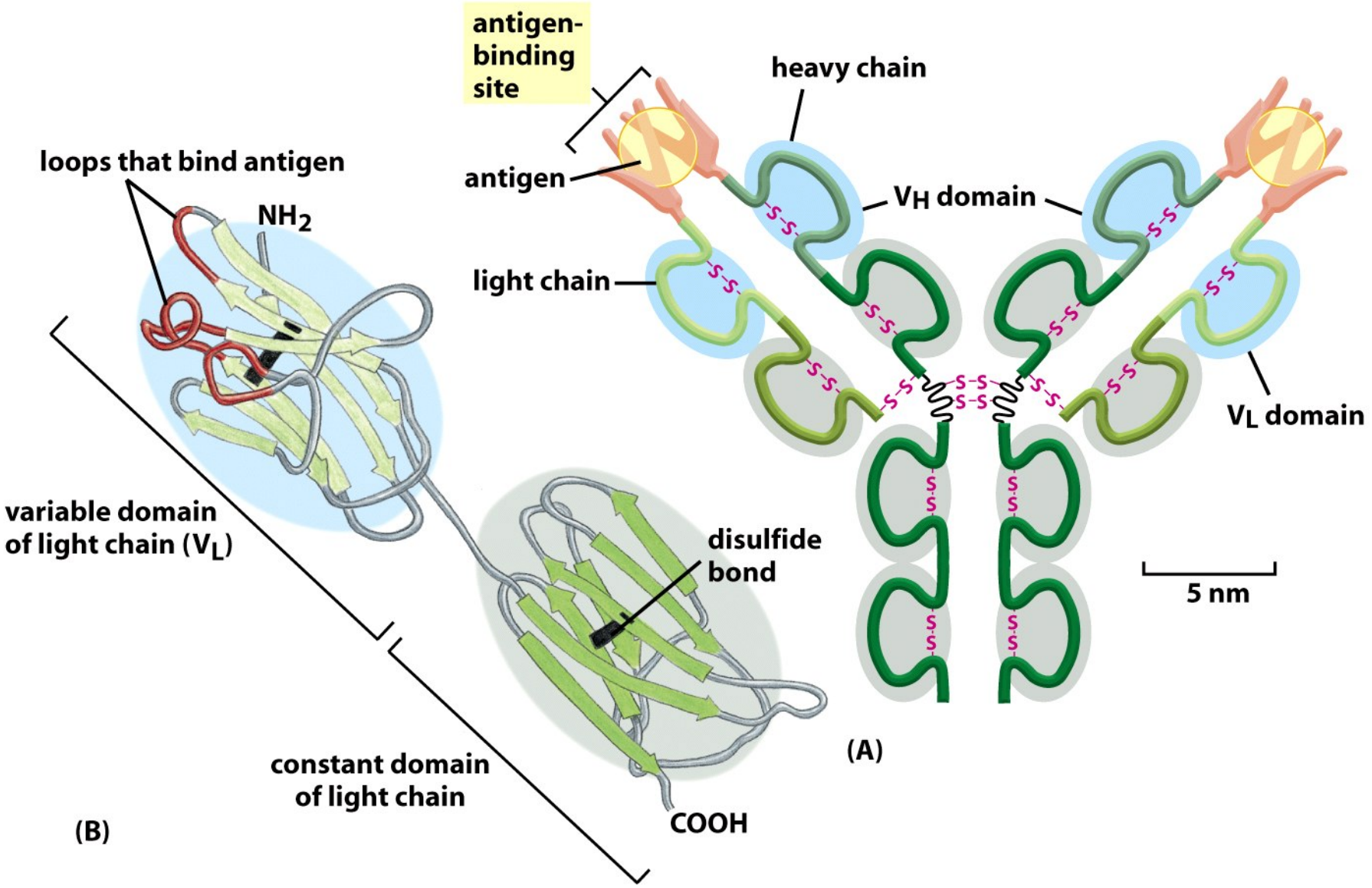
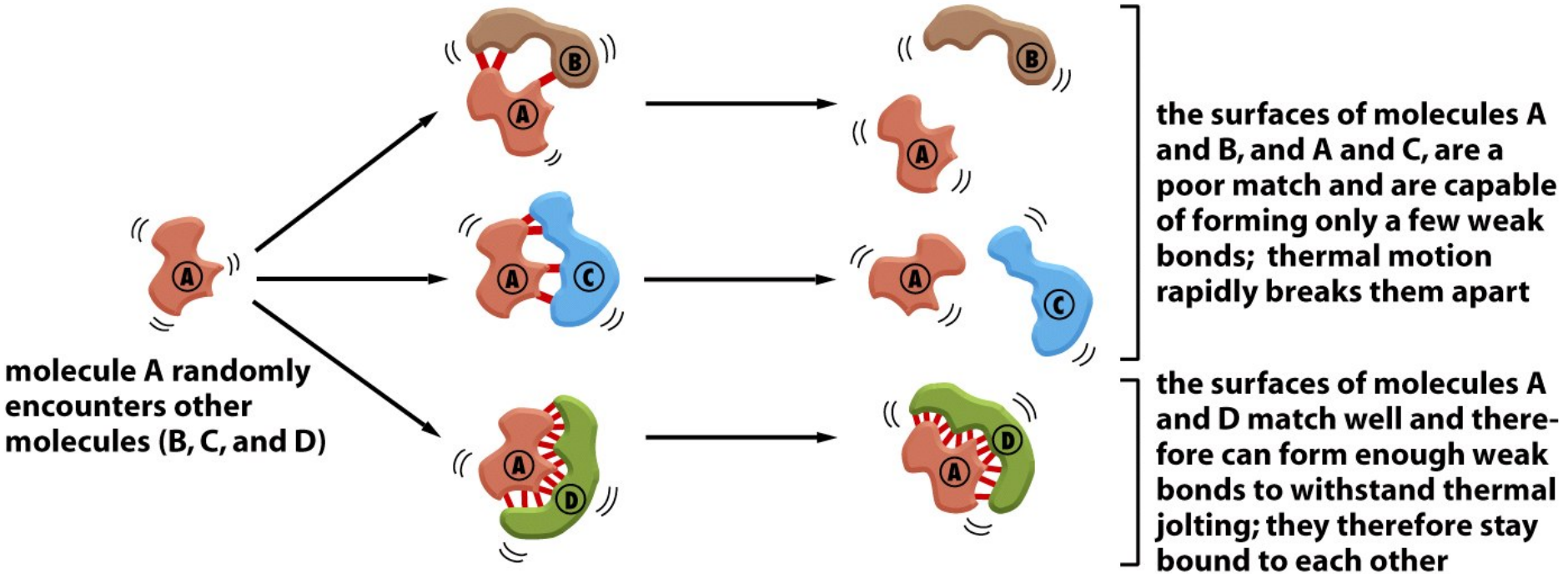
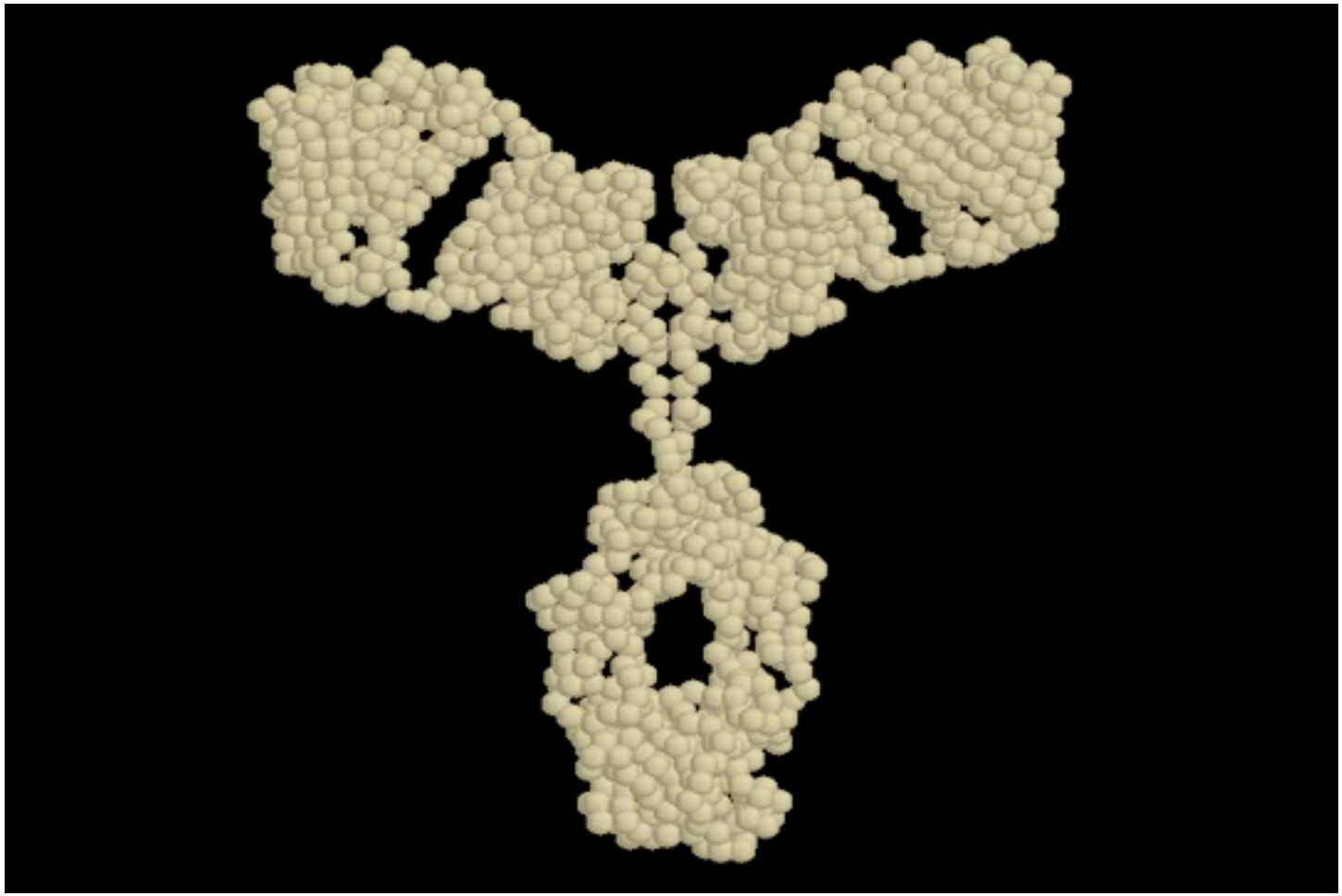
An antibody

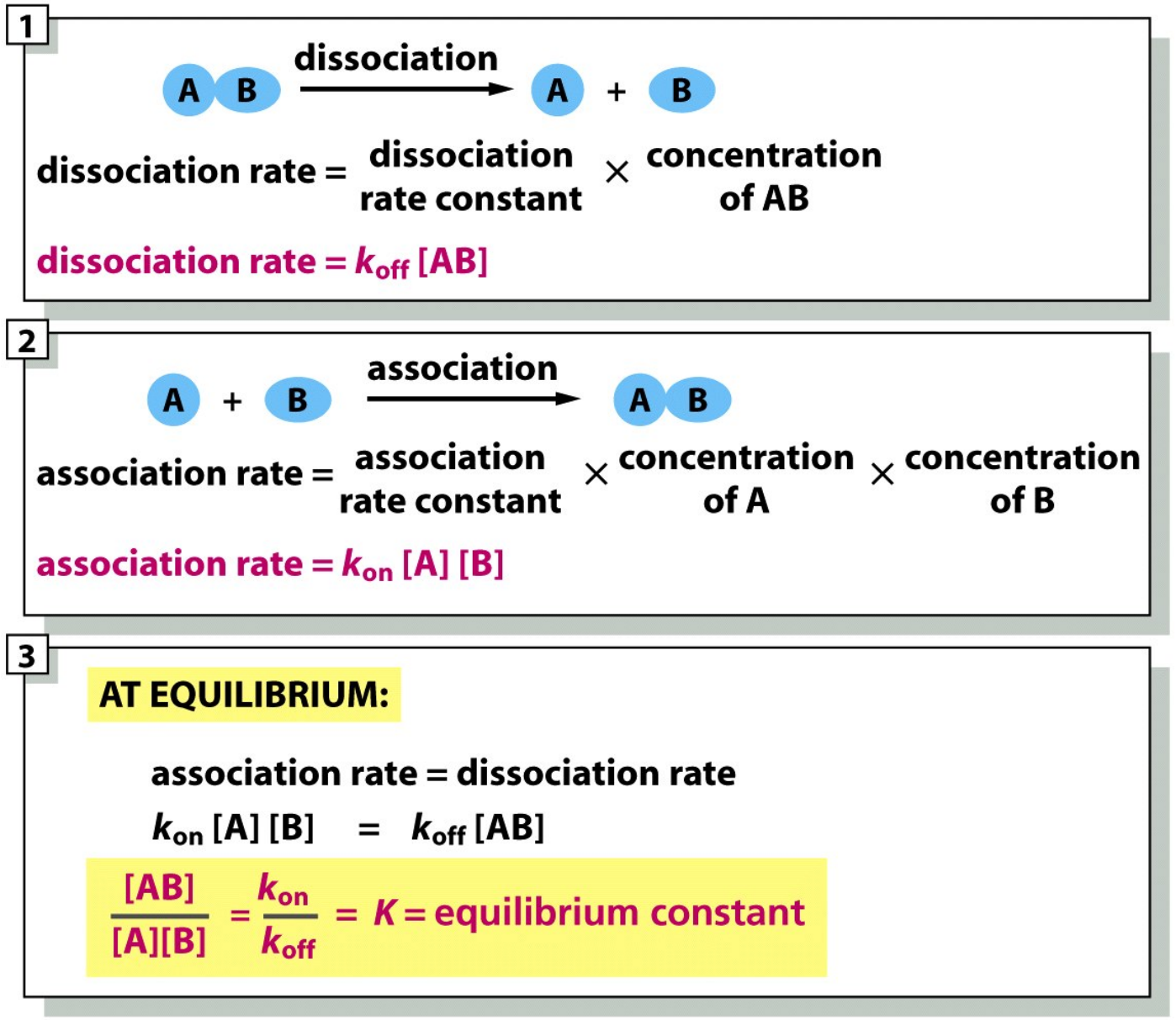


Interactions between molecules mediated by noncovalent bonds





Equilibrium constant for an association reaction



The relationship between free-energy differences and equilibrium constants (37°C)

equilibrium constant $\frac{[AB]}{[A][B]} = K$ (liters/mole)	free-energy difference of AB minus free energy of A + B (kcal/mole)	free-energy difference of AB minus free energy of A + B (kJ/mole)
1	0	0
10	−1.4	−5.9
10^2	−2.8	−11.9
10^3	−4.3	−17.8
10^4	−5.7	−23.7
10^5	−7.1	−29.7
10^6	−8.5	−35.6
10^7	−9.9	−41.5
10^8	−11.3	−47.4
10^9	−12.8	−53.4
10^{10}	−14.2	−59.4
10^{11}	−15.6	−65.3

Although joules and kilojoules (1000 joules) are standard units of energy, cell biologists usually refer to free energy values in terms of calories and kilocalories.

One kilocalorie (kcal) is equal to 4.184 kilojoules (kJ).

The relationship between the free-energy change, ΔG, and the equilibrium constant is

$$\Delta G = -0.00458\, T \log K$$

where ΔG is in kilocalories and T is the absolute temperature in Kelvins (310 K = 37°C).

Consider 1000 molecules of A and 1000 molecules of B in a eucaryotic cell. The concentration of both will be about 10^{-9} M.

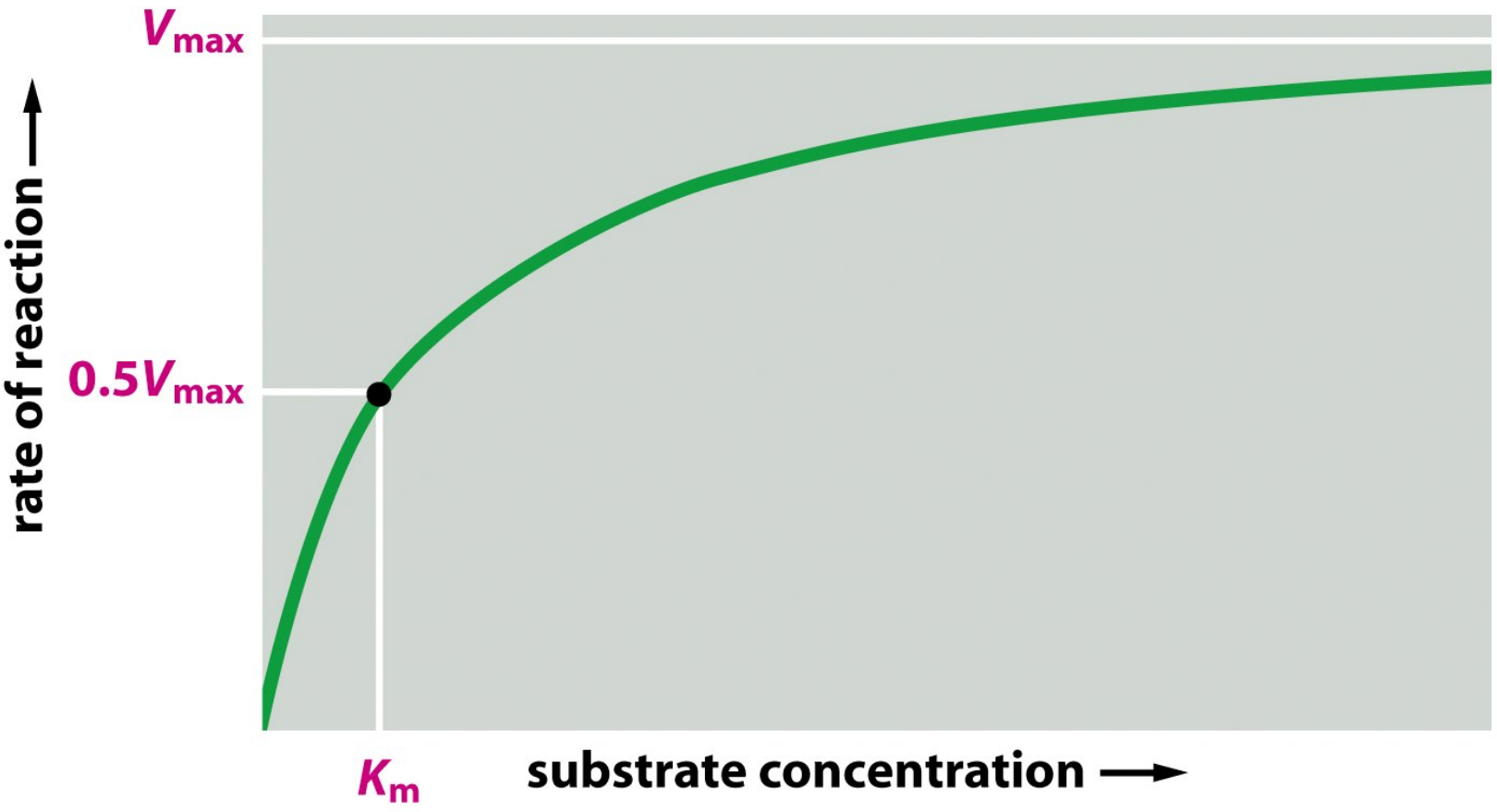
If the equilibrium constant (K) for A + B $\leftrightharpoons$ AB is 10^{10}, then at equilibrium there will be

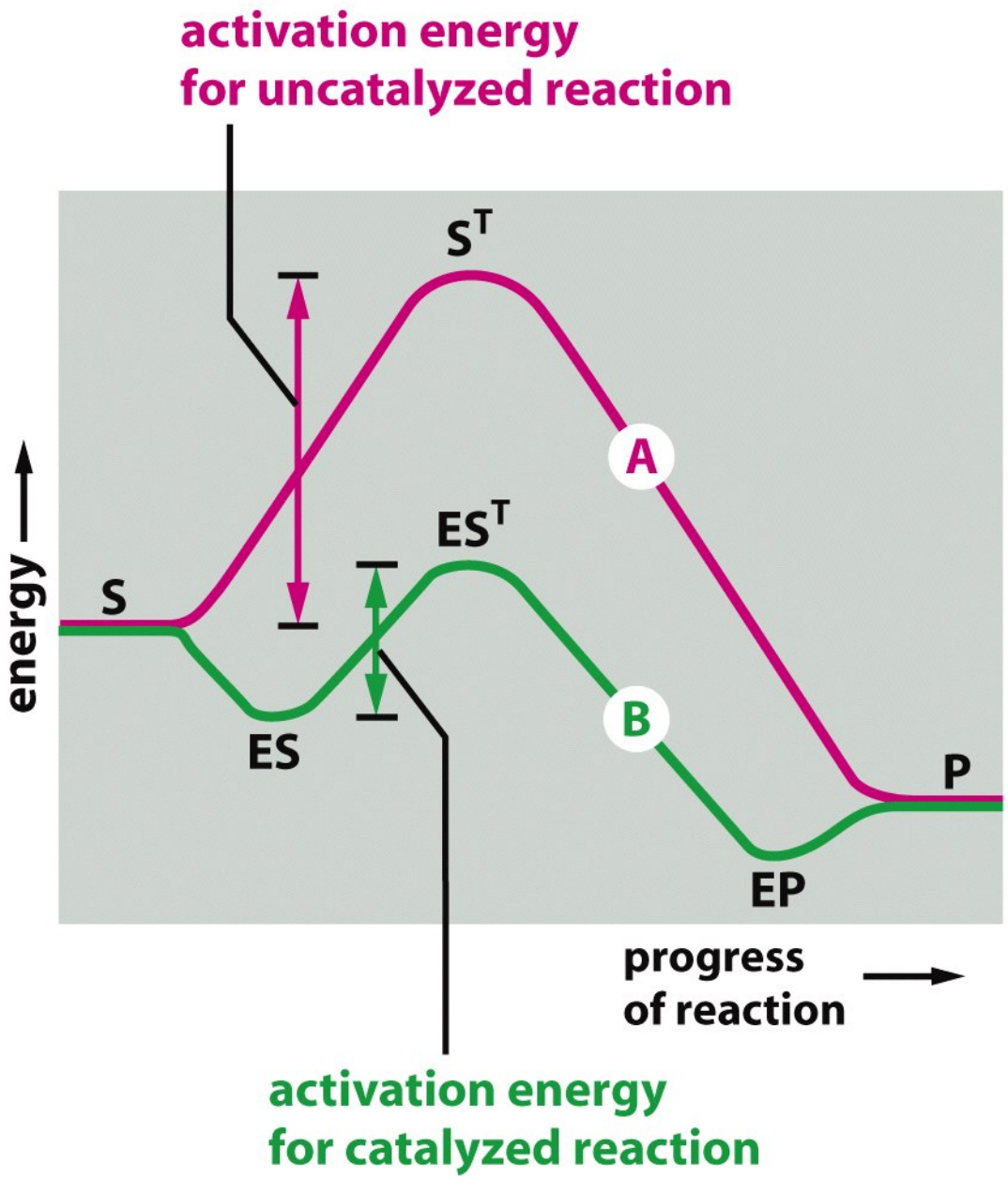
270	270	730
A molecules	B molecules	AB molecules

If the equilibrium constant is a little weaker at 10^{8}, which represents a loss of 2.8 kcal/mole of binding energy from the example above, or 2–3 fewer hydrogen bonds, then there will be

915	915	85
A molecules	B molecules	AB molecules

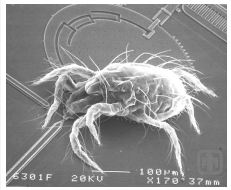
Enzyme kinetics





The Scale of Things – Nanometers and More

Things Natural



Dust mite

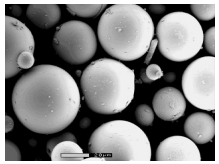
200 μm

Ant

~ 5 mm

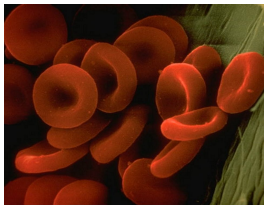
Human hair

~ 60-120 μm wide



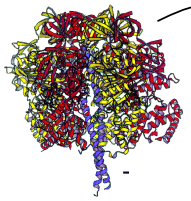
Fly ash

~ 10-20 μm

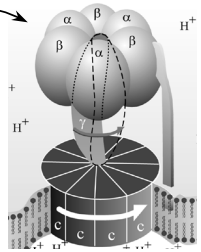


Red blood cells

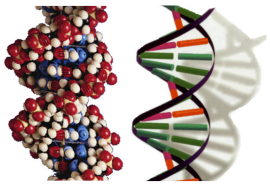
(~7-8 μm)



~10 nm diameter

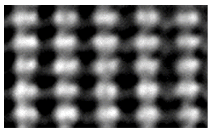


ATP synthase



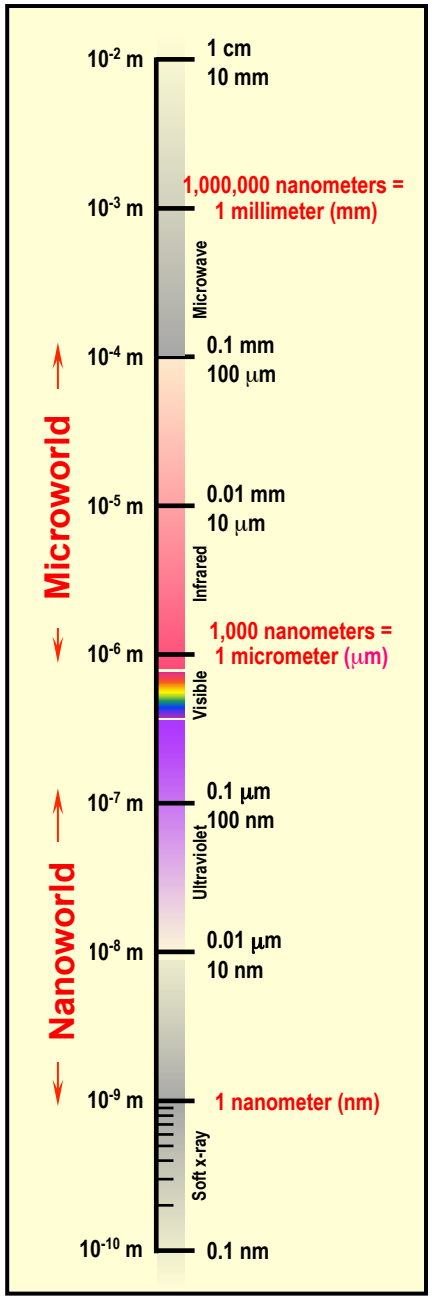
DNA

~2-1/2 nm diameter

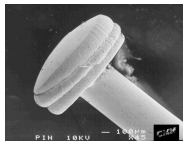


Atoms of silicon

spacing 0.078 nm

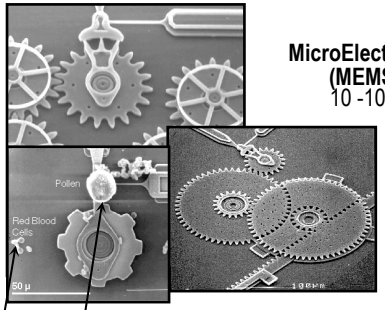


Things Manmade



Head of a pin

1-2 mm

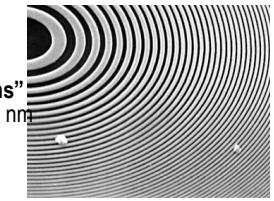


MicroElectroMechanical (MEMS) devices

10 -100 μm wide

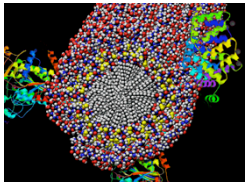
Pollen grain

Red blood cells



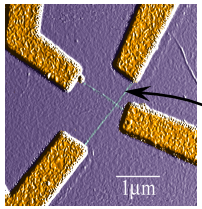
Zone plate x-ray "lens"

Outer ring spacing ~35 nm

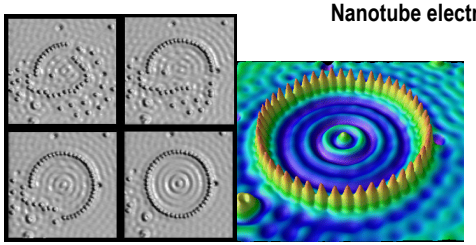


Self-assembled, Nature-inspired structure

Many 10s of nm

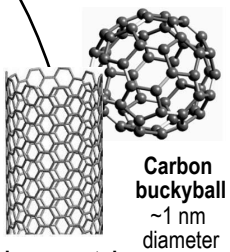


Nanotube electrode



Quantum corral of 48 iron atoms on copper surface positioned one at a time with an STM tip

Corral diameter 14 nm



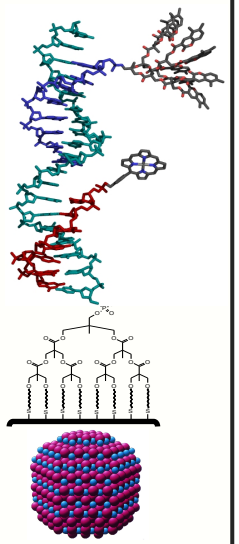
Carbon buckyball

~1 nm diameter

Carbon nanotube

~1.3 nm diameter

The Challenge



Fabricate and combine nanoscale building blocks to make useful devices, e.g., a photosynthetic reaction center with integral semiconductor storage.

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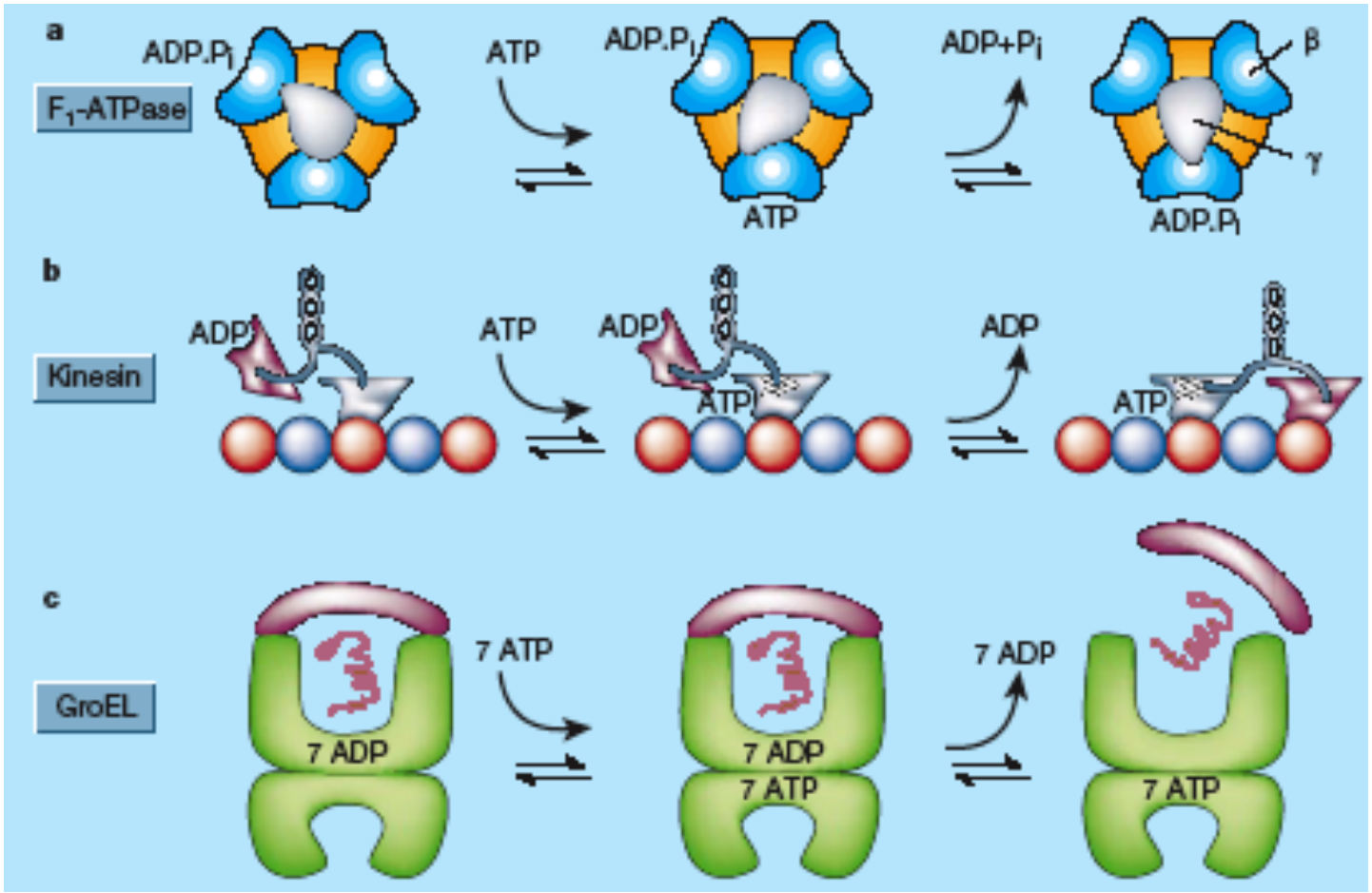
Office of Science, U.S. DOE
Version 05-26-06, pmd

Biomolecular motors

Biomotors convert chemical energy (ATP) to mechanical Energy which induces rotational or translational motion



Examples of biomolecular motors

- Cytoskeletal motors
- Polymerisation motors
- Rotary motors
- Nucleic acid motors
- Synthetic molecular motors

  (have been created by chemists that yield rotation, possibly generating torque)

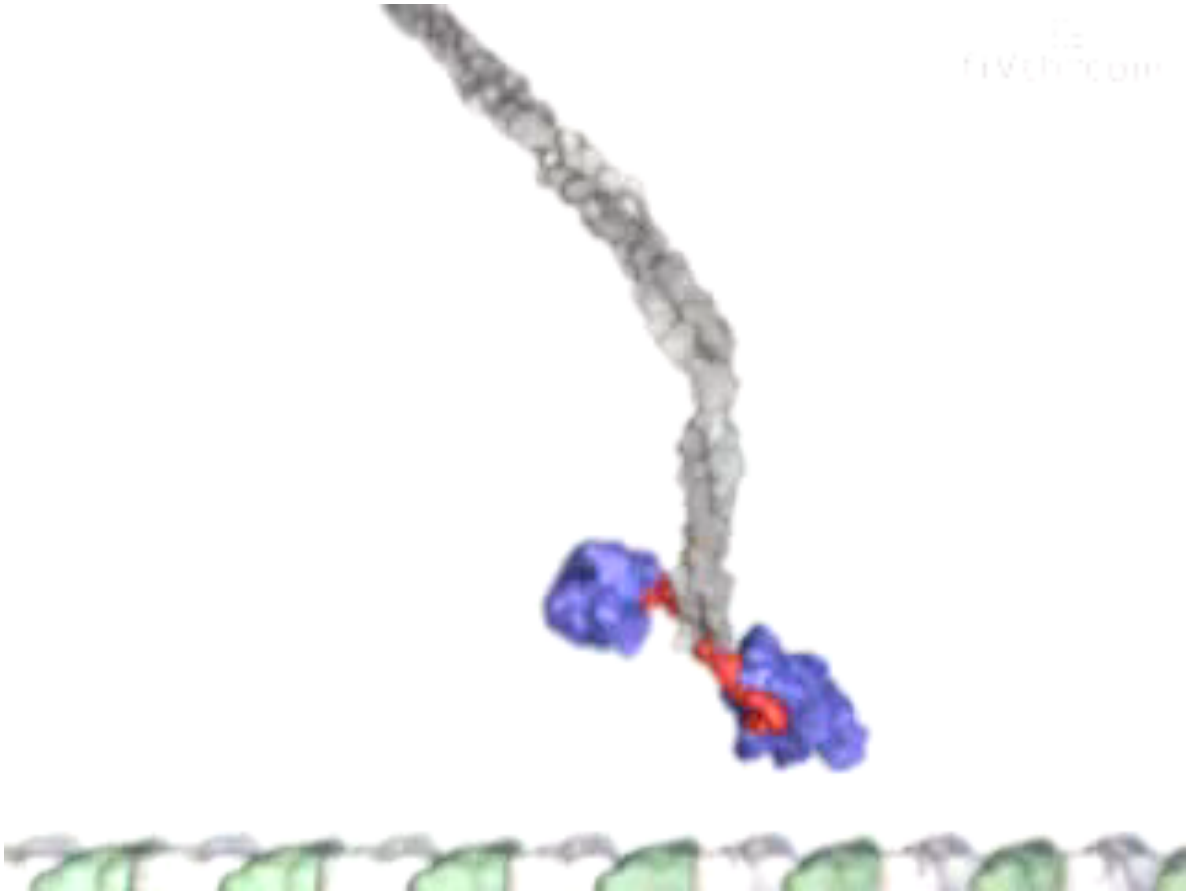
Cytoskeletal motors

- Myosin is responsible for muscle contraction
- Kinesin moves cargo inside cells away from the nucleus along microtubules
- Dynein produces the axonemal beating of cilia and flagella and also transports cargo along microtubules towards the cell nucleus

Schematic representation of an eukaryotic cell, showing the actin (red lines) and microtubule (blue lines) cytoskeleton and different types of the motor proteins.

http://www.motorprotein.de/research.html

Kinesin–microtubule



Vesicle tracking along the tubulin network in a living cell (movie)

Polymerisation motors

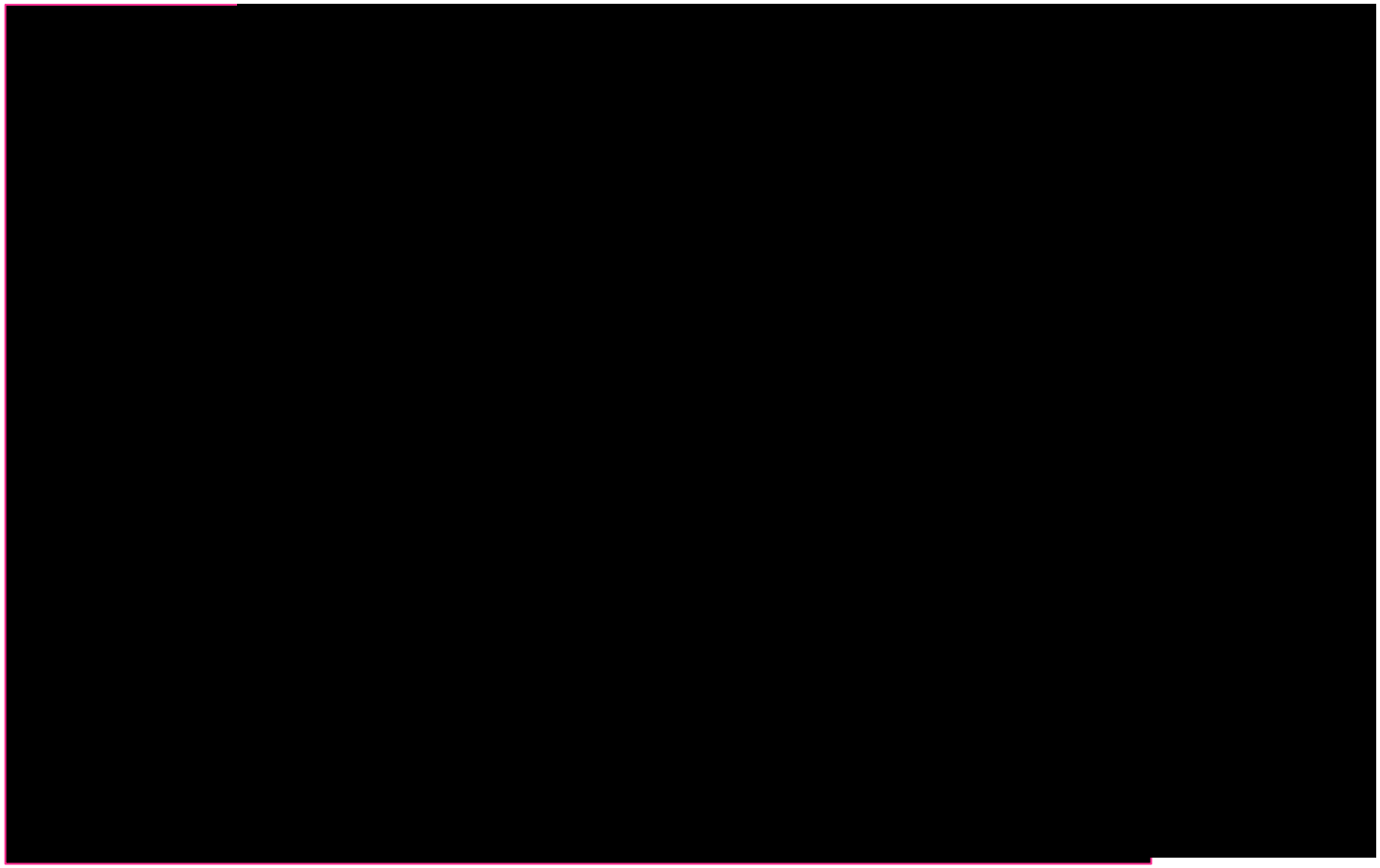
- Actin polymerization generates forces and can be used for propulsion. ATP is used.
- Microtubule polymerization using GTP.
- Dynamin is responsible for the separation of clathrin buds from the plasma membrane.

Nucleic acid motors:

- RNA polymerase transcribes RNA from a DNA template
- DNA polymerase turns single-stranded DNA into double-stranded DNA
- Nucleic acid double strand separation prior to transcription or replication (helicase)
- Topoisomerases reduce supercoiling of DNA in the cell
- chromatin remodeling (RSC complex)
- chromosome condensation (SMC protein)
- Viral DNA packaging motors inject viral genomic DNA into capsids as part of their replication cycle, packing it very tightly

DNA Replication Process-DNA polymerase

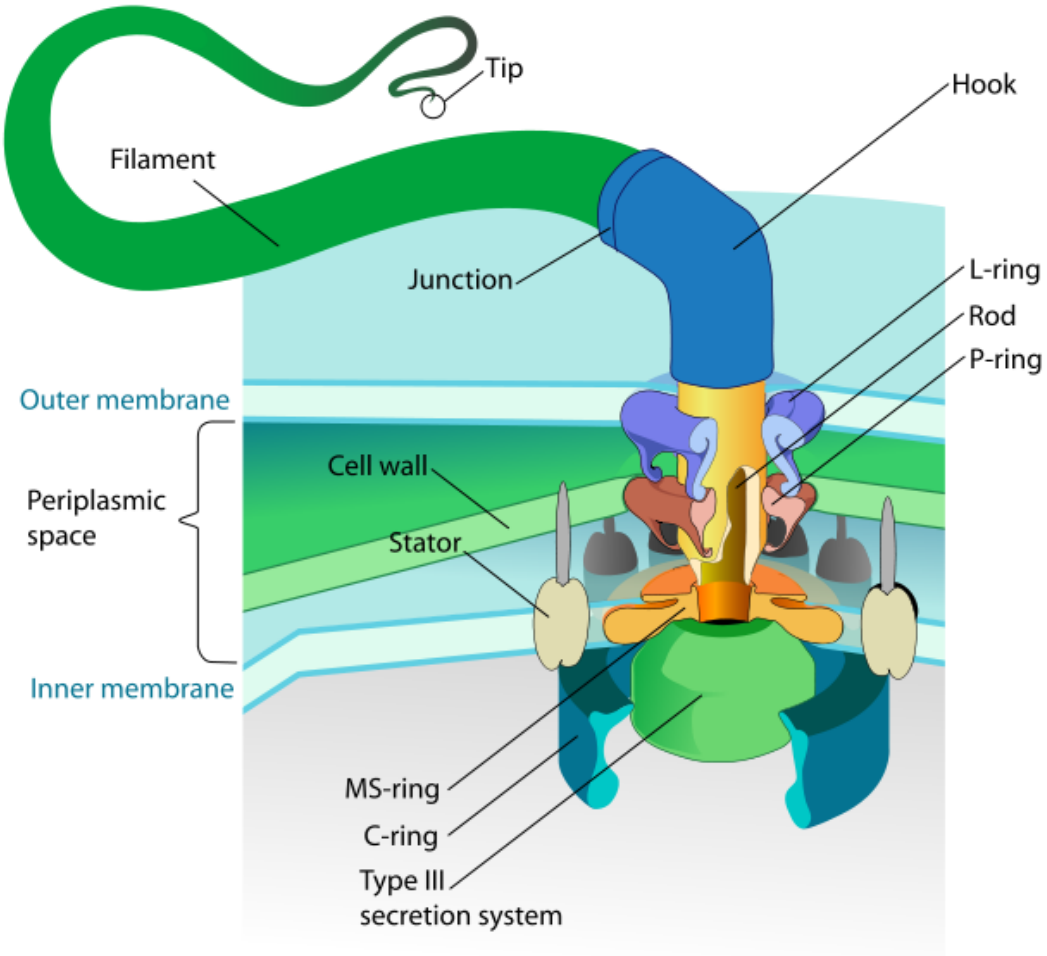
DNA Replication Process-helicase



Rotary motors:

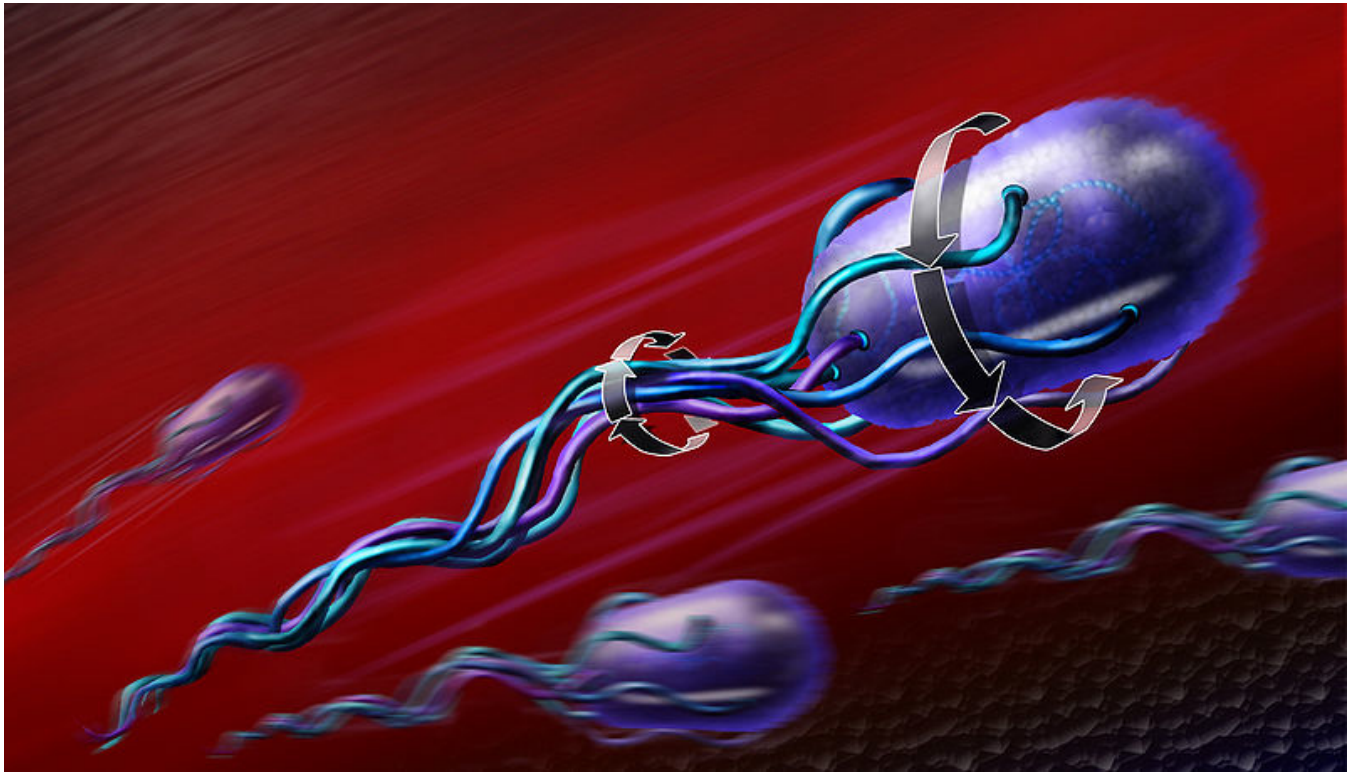
- FoF1-ATP synthase generates ATP using the transmembrane electrochemical proton gradient inside mitochondria
- The bacterial flagellum responsible for the swimming and tumbling of E. coli and other bacteria acts as *a rig*id propeller that is powered by a rotary motor. This motor is driven by the flow of protons across a membrane, possibly using a similar mechanism to that found in the Fo motor in ATP synthase.

Bacterial flagellum

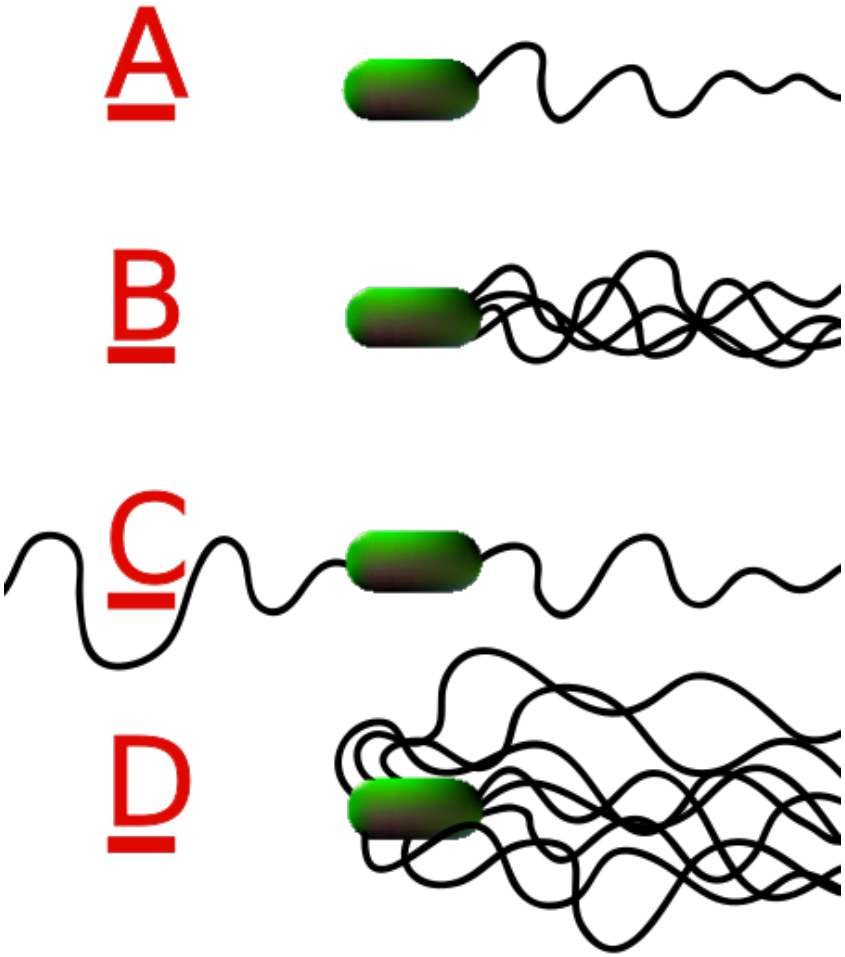


A Gram-negative bacterial flagellum. A flagellum (plural: flagella) is a long, slender projection from the cell butt body, whose function is to propel a unicellular or small multicellular organism. *The bacterial movement can be divided in 2 kinds: run, resulting from a counterclockwise rotation of the flagellum, and tumbling, from a clockwise rotation of the flagellum.*

Bacterial flagellum

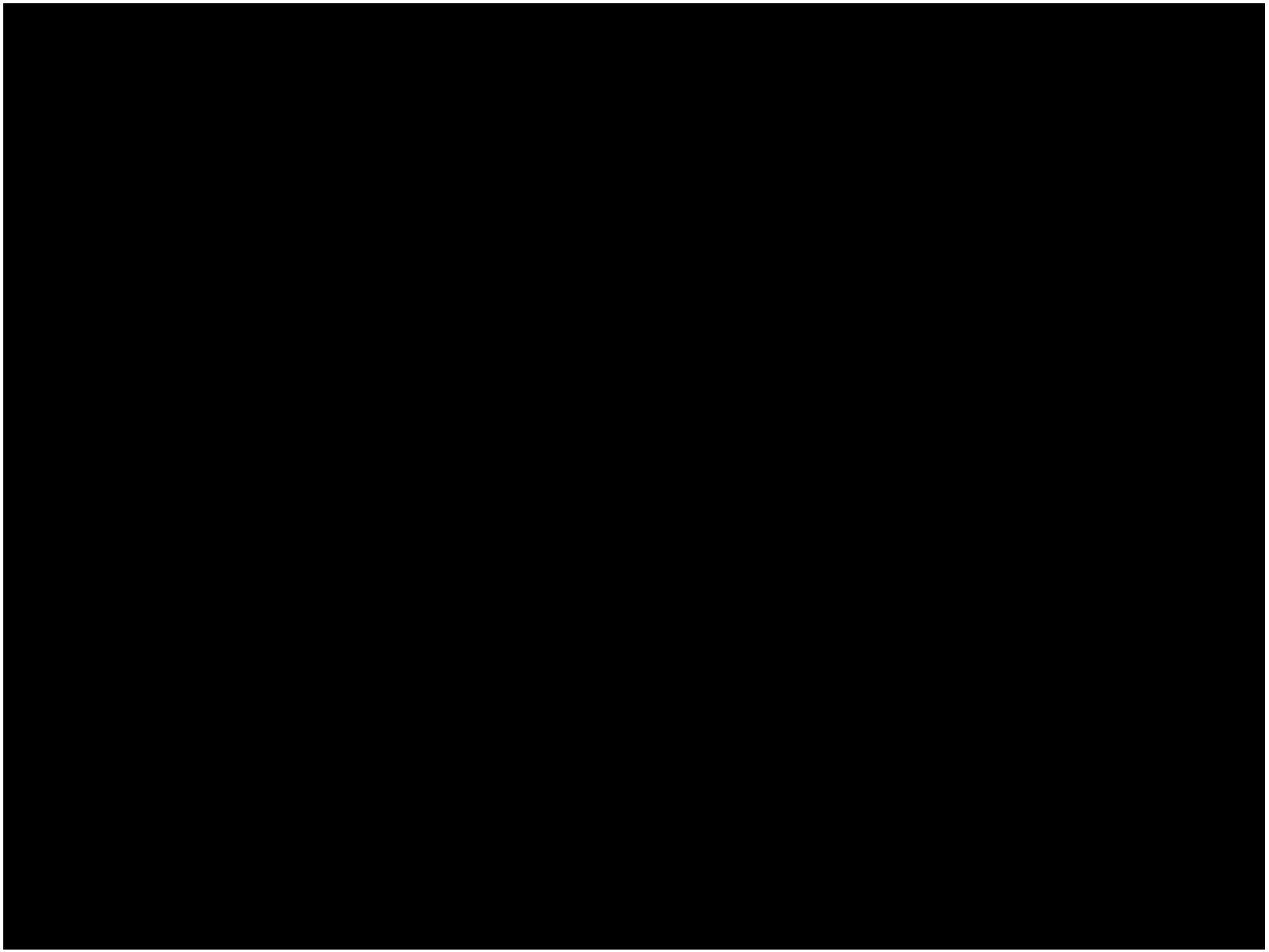


Escherichia coli cells use long, thin structures called flagella to propel themselves. These flagella form bundles that rotate counter-clockwise, creating a torque that causes the bacterium to rotate clockwise.

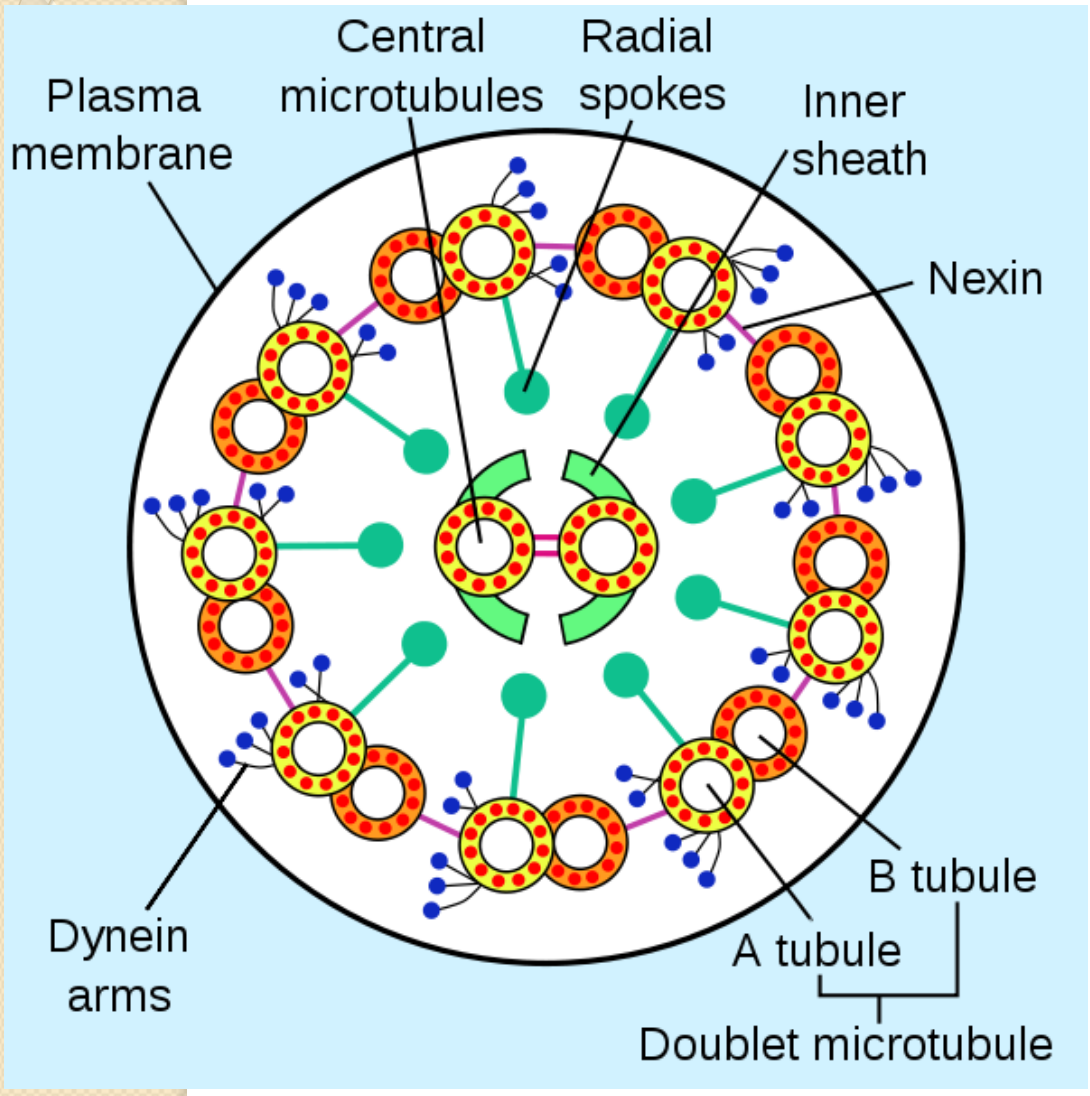


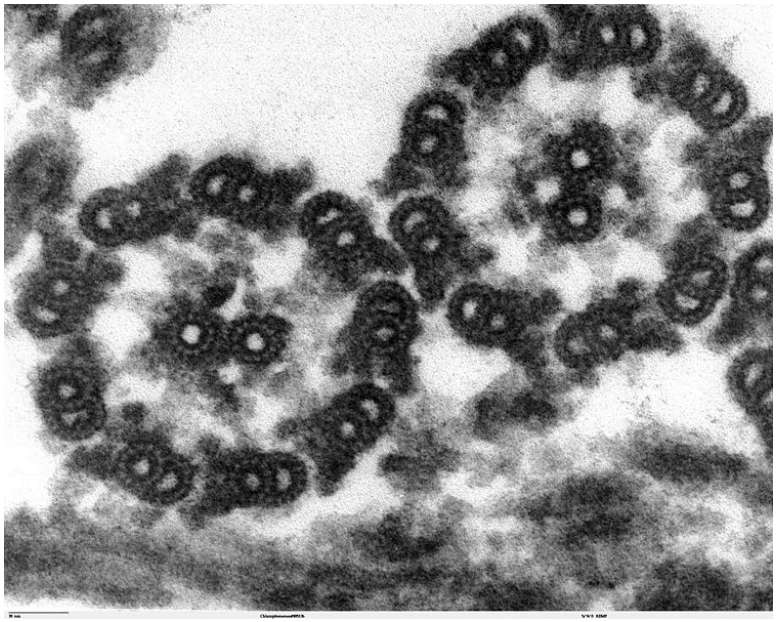
- Examples of bacterial flagaella arrangment schemes. A-Monotrichous; B-Lophotrichous; C-Amphitrichous; D-Peritrichous

Bacterial flagellum-animation



A cross sectional diagram through a typical eukaryotic flagellum showing the 9+2 arrangement of microtubules

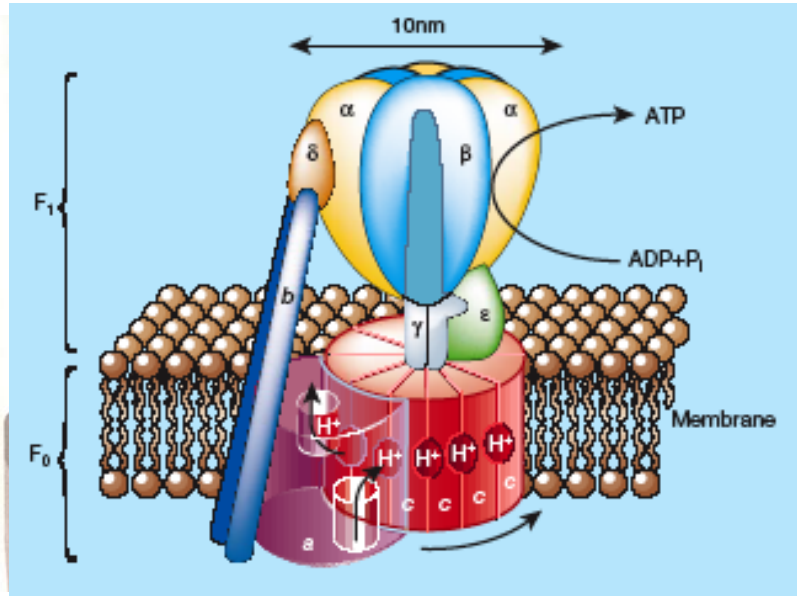




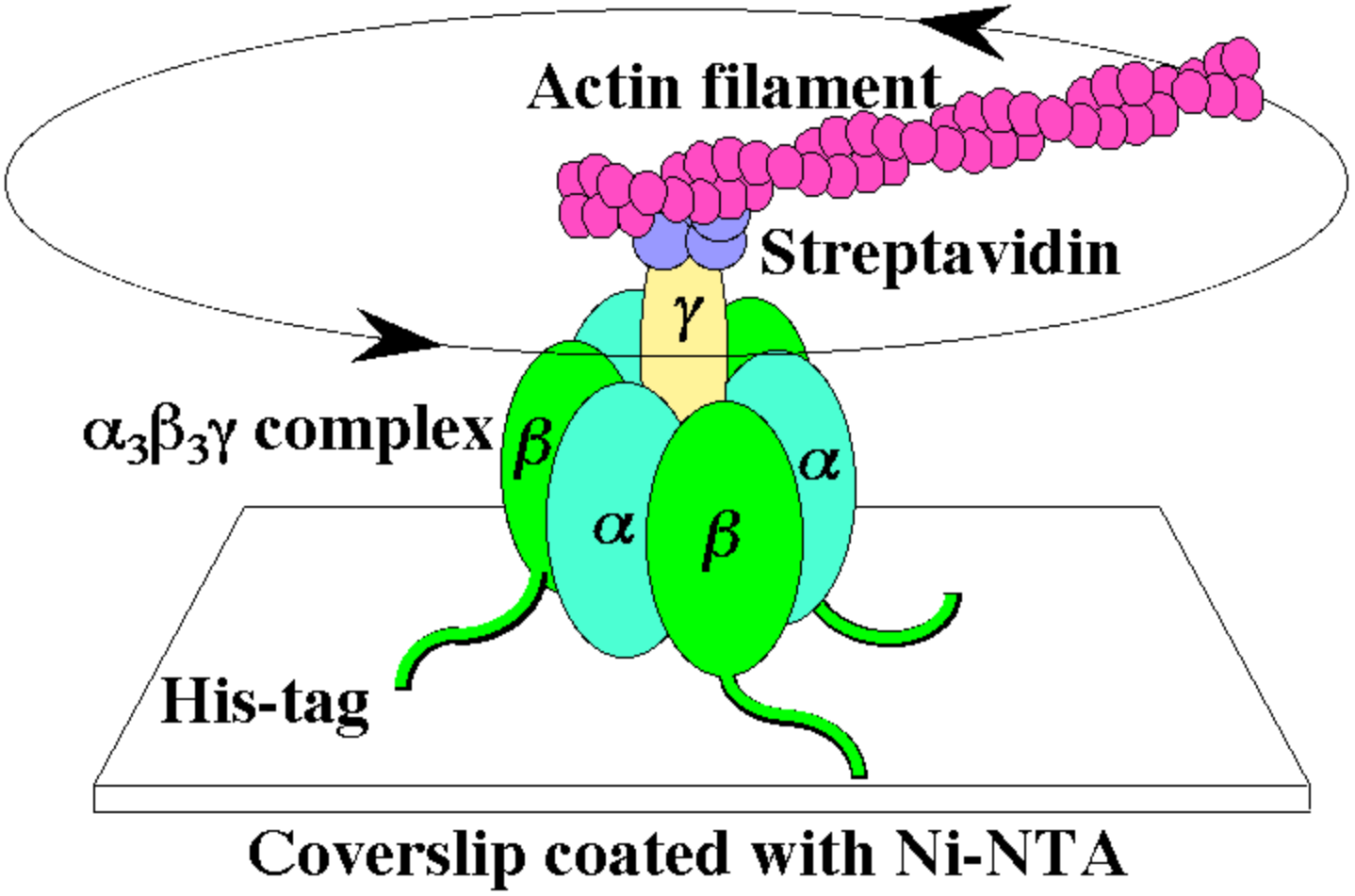
The "9+2" structure is visible in this cross-section TEM of axoneme

FoF1-ATP synthase

- ATPase: Produce and hydrolyze ATP
- F1 Complex: $\alpha_3\beta_3$ hexamer and γ subunit
- γ subunit (> 12 nm) rotates due to the conversion of chemical to mechanical energy (80-100% efficiency).

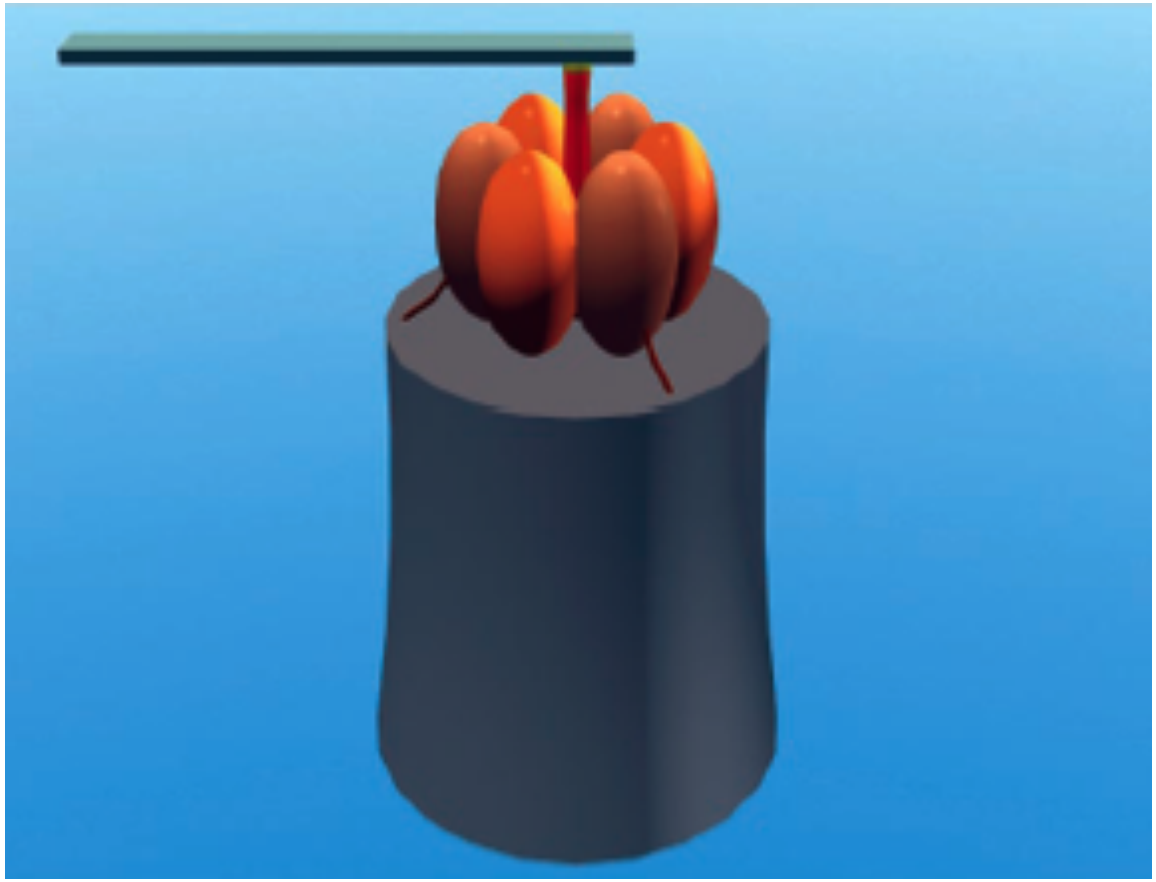


Attachment of Actin Filaments



Hybrid organic-inorganic nanobiodevice

Nanopropeller

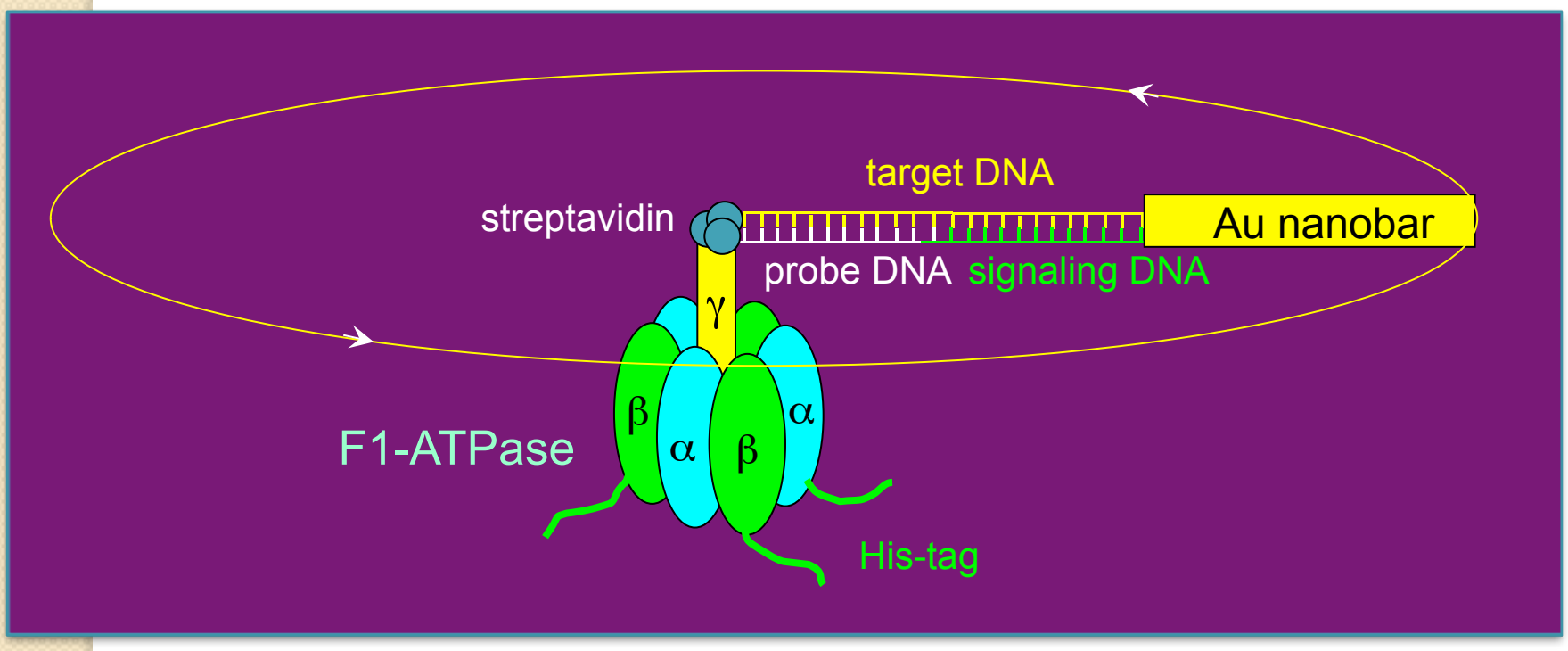


Soong et al., Science 290, 1555 (2000)

Biomotor-based single molecular sensors

Detecting Specific DNA Sequences

"Molecular Semaphore"



C.F. Chou *et al.*, US patent 6,989,235 (2006)

F1-ATPase biomotor

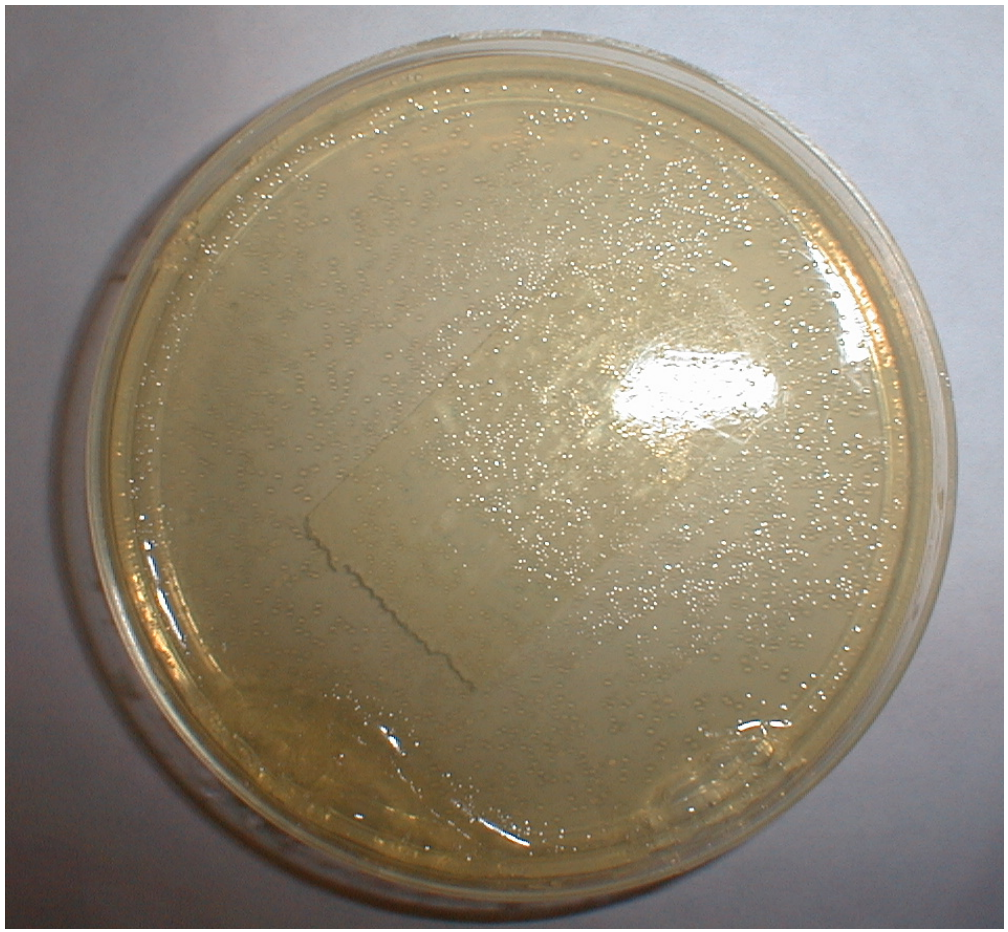
- Establish the Cell-line for the production of nanomotor
- Developed and evaluated cell lysis and protein extraction procedure
- Developed and optimized Nanomotor purification procedure
- Developed and tested protein concentration assay and established standard curve using BSA
- Developed ATPase assay and demonstrated ATPase activity of purified ATPase nanomotor

Outline of the Processes

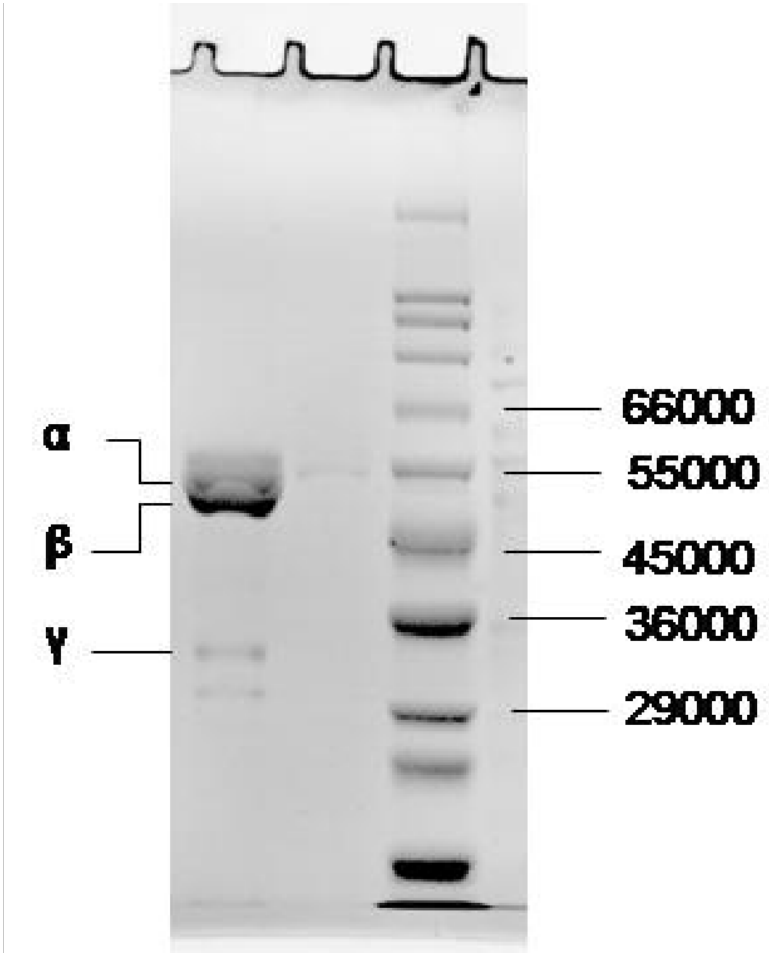
1. Cell Culturing (production of ATPase)
2. Coating with Ni
3. Construction of observation chamber
4. Synthesis of bar-shaped objects
5. Immobilization of the motor
6. Attachment of objects to the motor
7. Observation of the rotation

Cell Culturing

Cells carrying the motor have been cultured.

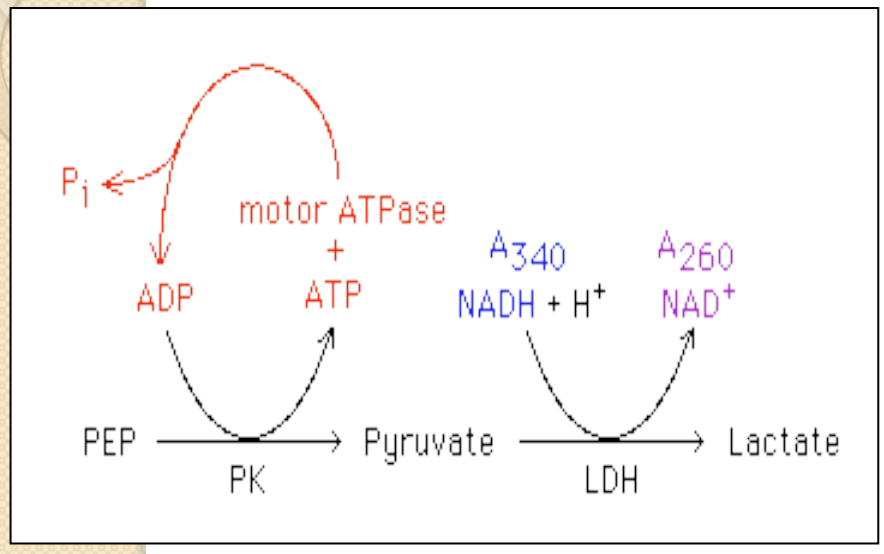


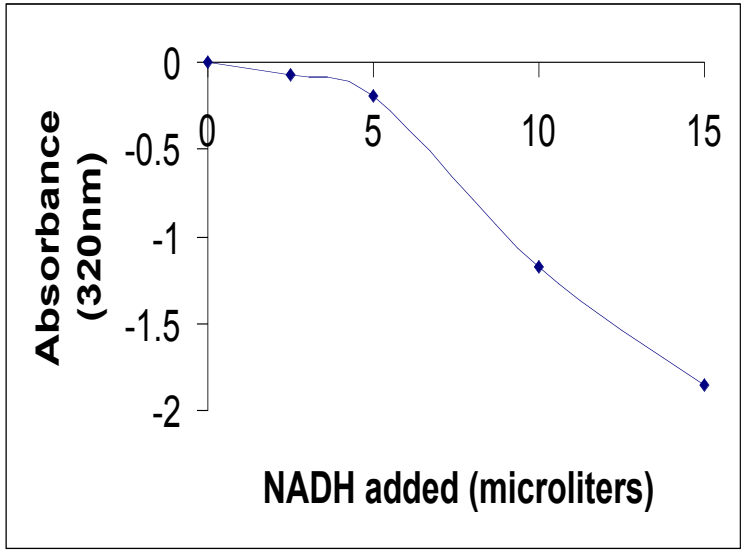
Purification of the recombinant $\alpha_3\beta_3\gamma$ complex



Purified sample and protein markers were analyzed on 12% PAGE (12%) Gels were stained with Coomassie brilliant blue.

F1-ATPase motility assay

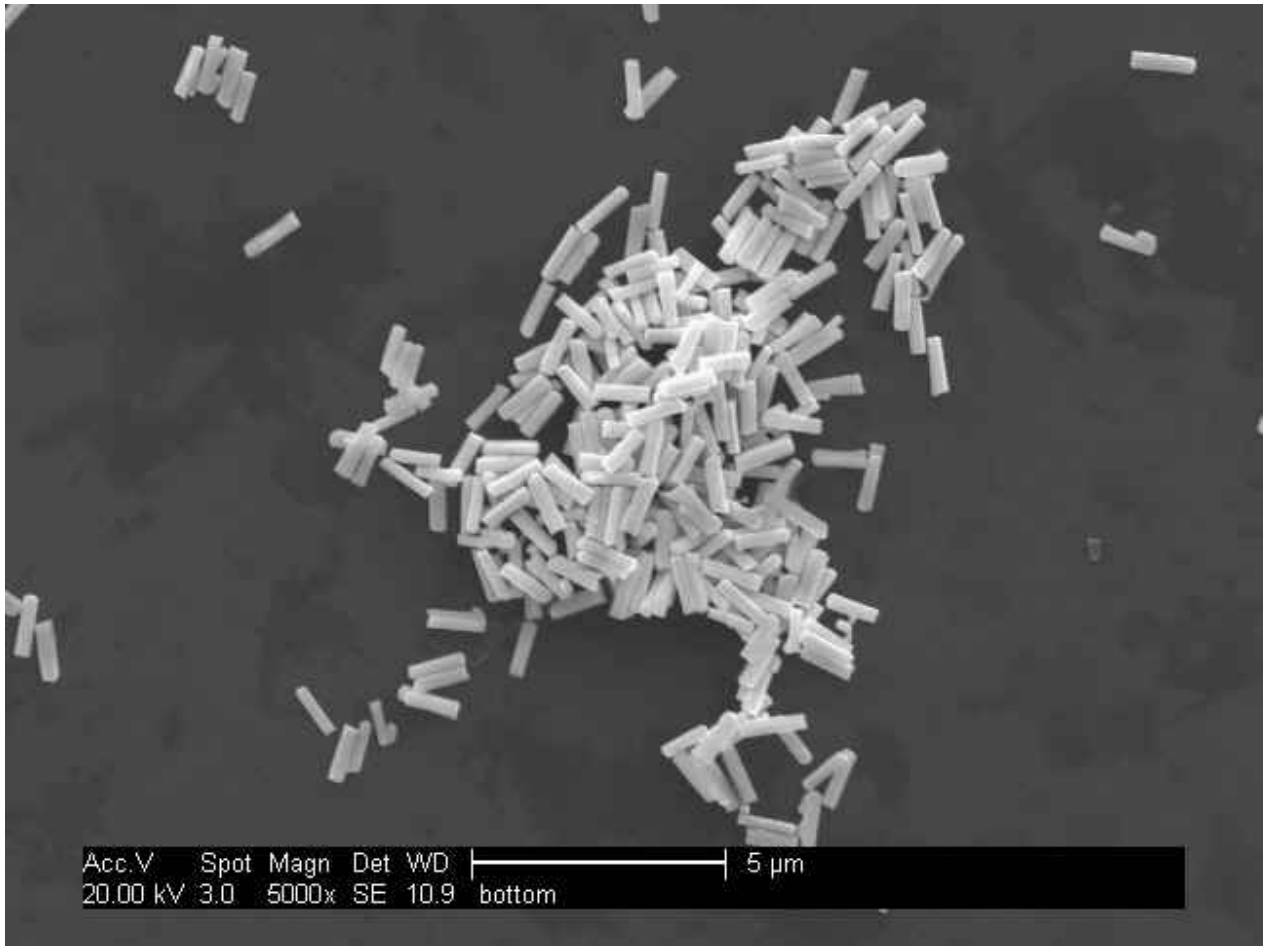




ATP regeneration assay.
The addition of motor ATPase causes the oxidation of NADH to NAD^+ and the decrease of absorbance at 340nm.

The effect of the amount of NADH on the ATP regeneration assay.
The increased absorbance drop at 320nm agrees with the increased amount of NADH added.

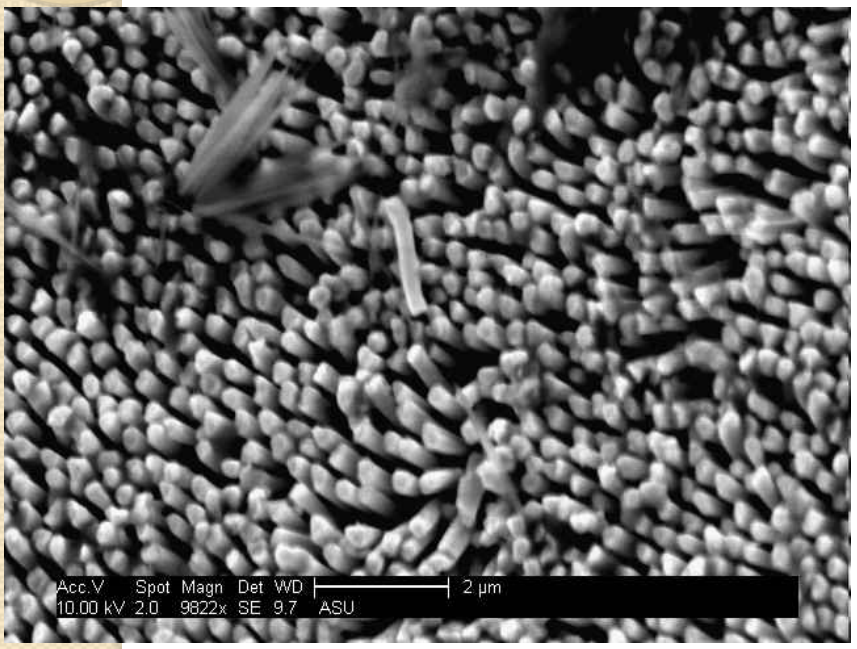
Au nanorods for biomotor conjugation



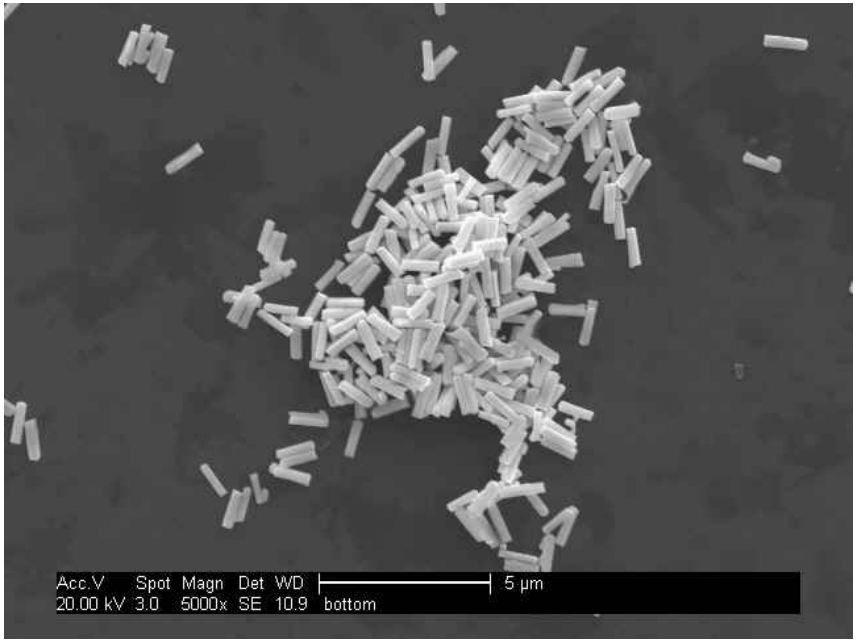
Au nanorods (1 µm long, 200 nm in diameter)

(Nanoplex)

Synthesis of nanoparticles for biomotor conjugation (electrochemical deposition using alimina templates)



Ni nanobars



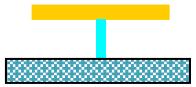
Au nanobar

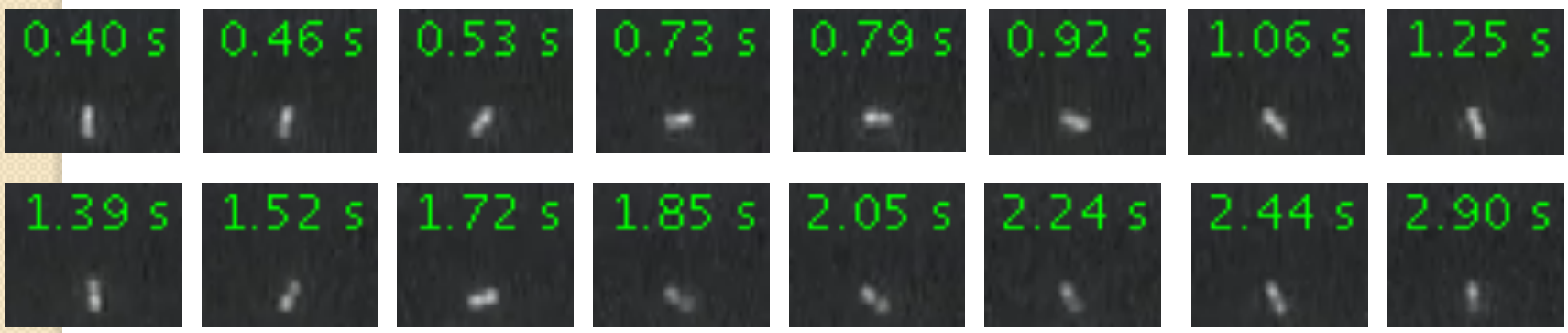
(both are 2 µm long and 200 nm in dia.)

Single F_1-ATPase rotation through Au nanobar conjugation

Brownian motion of Non-bound Au nanobars

Conjugation to F_1-ATPase at the center of streptavidin-coated Au rod





Observations were made using 100x oil immersion lens with bright field and were captured with a video camera (frame rate 30 Hz).