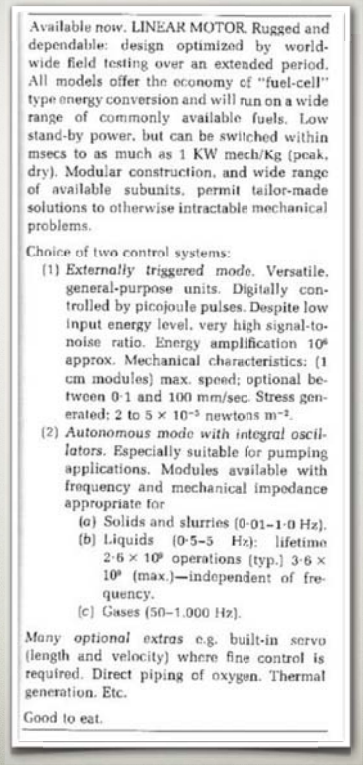
Muscle biophysics

Notice of a lecture presented by Professor D.R. Wilkie to the Institution of Electrical Enginers in London.



Available now. LINEAR MOTOR. Rugged and dependable: design optimized by world-wide field testing over an extended period. All models offer the economy of "fuel-cell" type energy conversion and will run on a wide range of commonly available fuels. Low stand-by power, but can be switched within msecs to as much as 1 KW mech/Kg (peak, dry). Modular construction, and wide range of available subunits, permit tailor-made solutions to otherwise intractable mechanical problems.

Choice of two control systems:

(1) *Externally triggered mode.* Versatile, general-purpose units. Digitally controlled by picojoule pulses. Despite low input energy level, very high signal-to-noise ratio. Energy amplification 10^6 approx. Mechanical characteristics: (1 cm modules) max. speed; optional between 0·1 and 100 mm/sec. Stress generated; 2 to 5×10^{-5} newtons m^{-2}.

(2) *Autonomous mode with integral oscillators.* Especially suitable for pumping applications. Modules available with frequency and mechanical impedance appropriate for

(a) Solids and slurries (0·01–1·0 Hz).

(b) Liquids (0·5–5 Hz): lifetime $2·6 \times 10^9$ operations (typ.) $3·6 \times 10^9$ (max.)—independent of frequency.

(c) Gases (50–1,000 Hz).

Many optional extras e.g. built-in servo (length and velocity) where fine control is required. Direct piping of oxygen. Thermal generation. Etc.

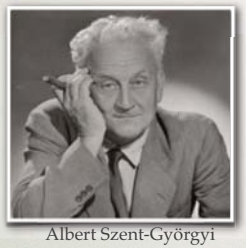
Good to eat.

Muscle

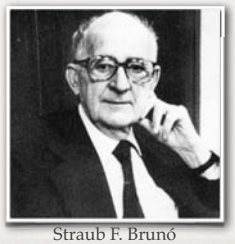
Tissue and/or cell specialized for the generation of force and movement.

It can only pull, not push (...).

Hungarians in muscle research



Albert Szent-Györgyi



Straub F. Brunó

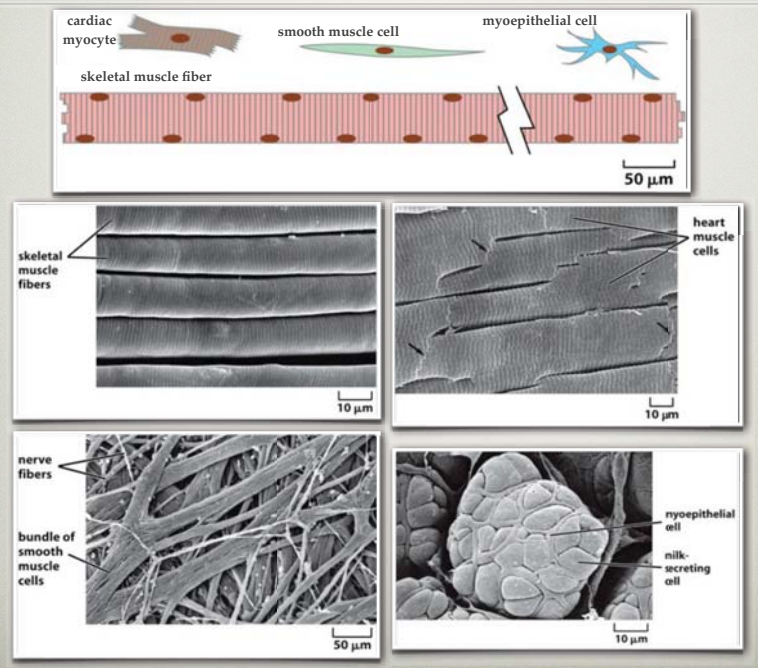
András Szent-Györgyi

János Gergely

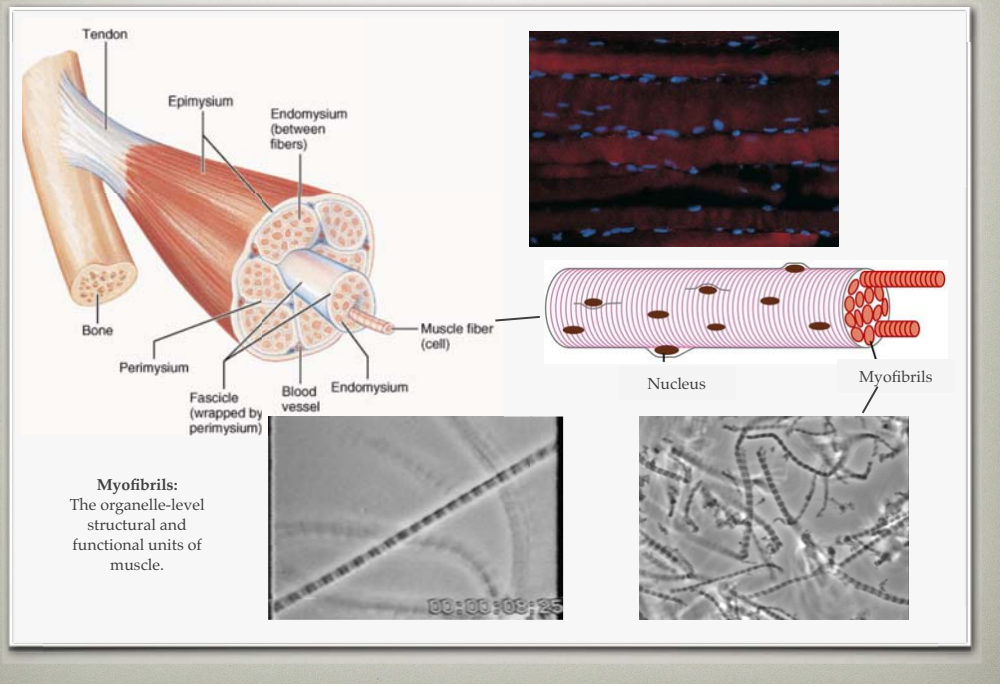
Katalin and Mihály Bárány

Ferenc Guba (fibrillin)

Types of muscle



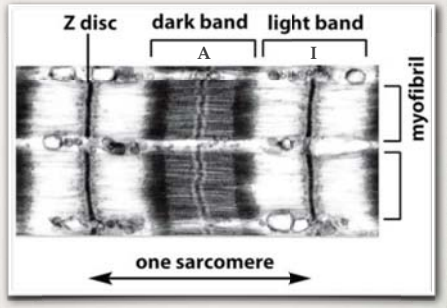
Skeletal muscle

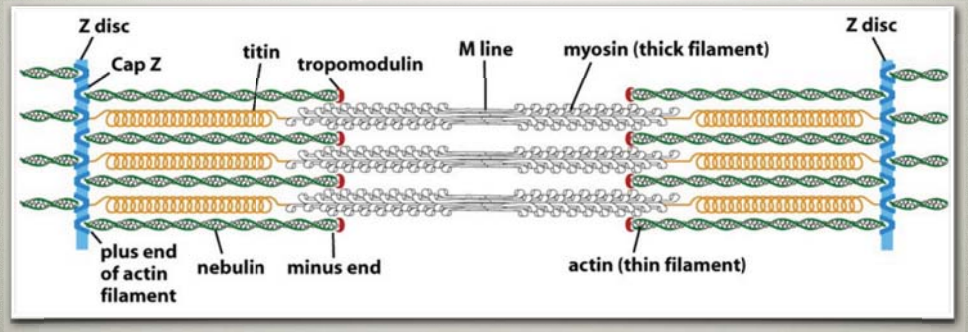


Myofibrils:
The organelle-level structural and functional units of muscle.

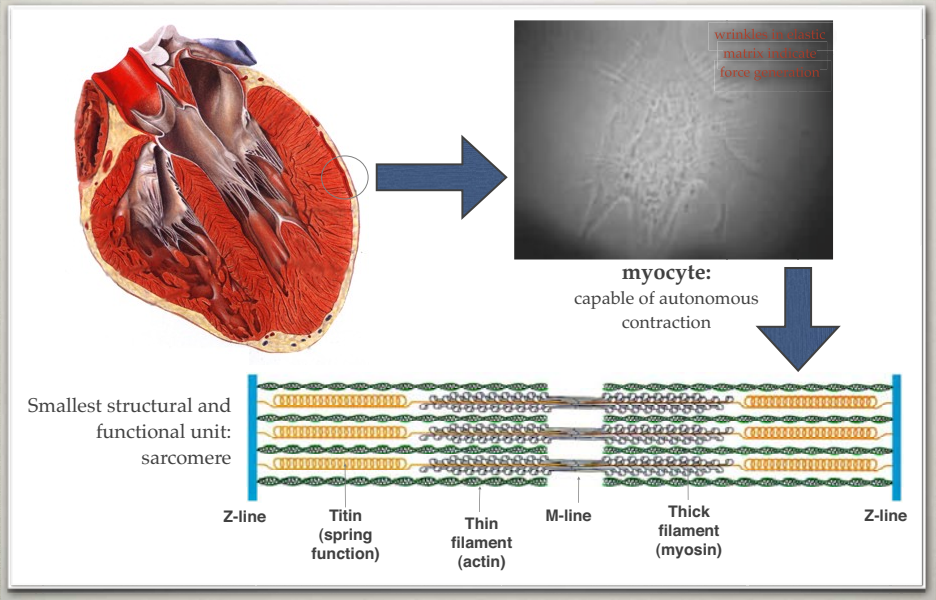
The sarcomere

sarcos: meat (Gr)
mera: unit
the smallest structural and functional unit of striated muscle.





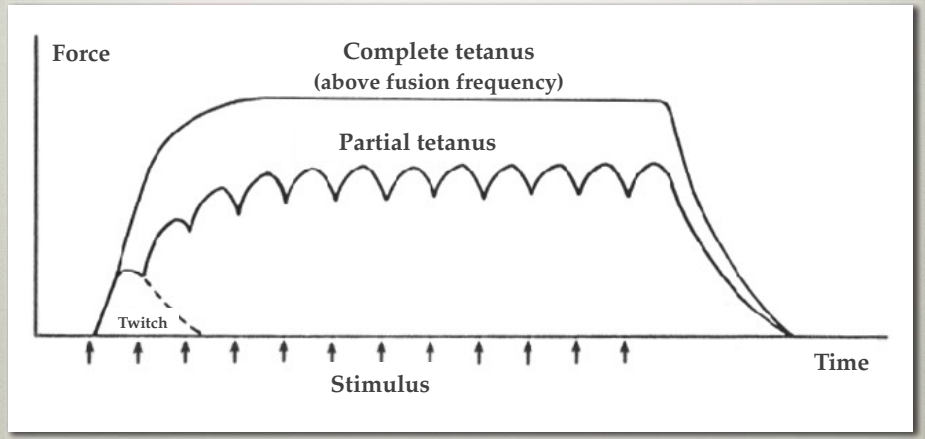
Heart muscle



myocyte:
capable of autonomous contraction

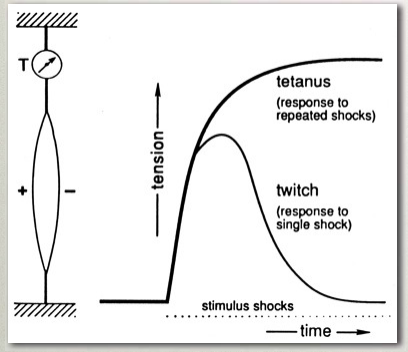
Smallest structural and functional unit: sarcomere

Basic phenomena of muscle function I.

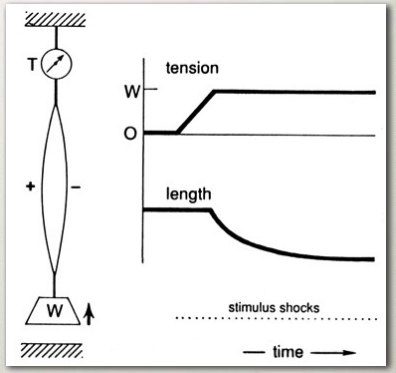


Basic phenomena of muscle function II.

1. Isometric contraction



2. Isotonic contraction



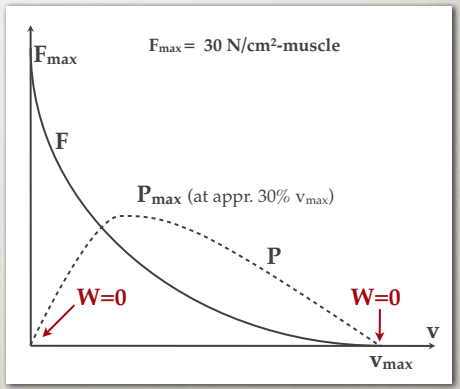
Auxotonic contraction (simultaneous shortening and force generation)

Basic phenomena of muscle function III.

1. Work, Power

$$W = Fs$$
$$P = Fs/t = Fv$$

2. Force-velocity diagram

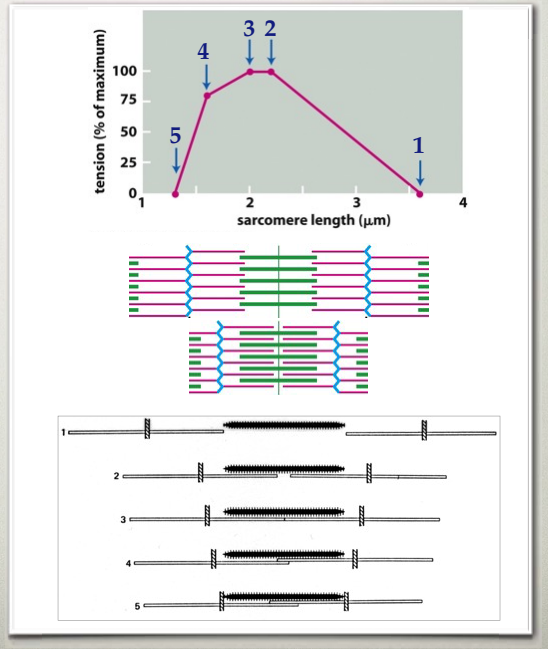


Mechanisms of muscle shortening

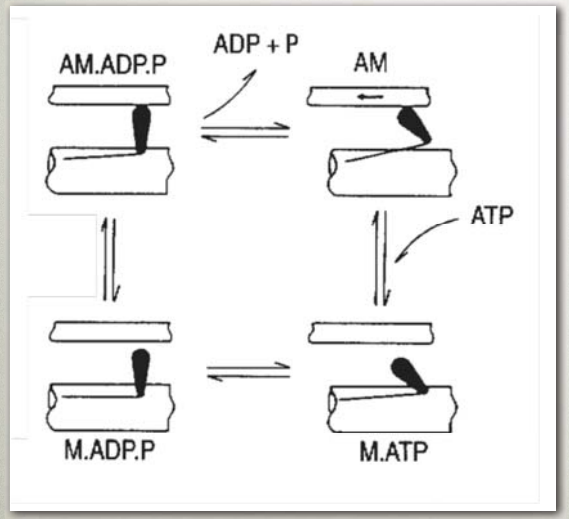
Phenomenological mechanism:

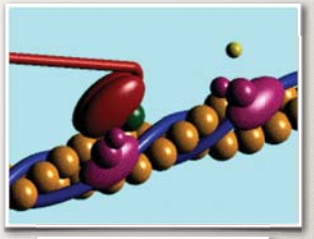
Sliding filament theory

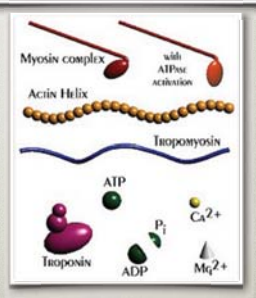
Andrew F. Huxley, Jean Hanson, Hugh E. Huxley



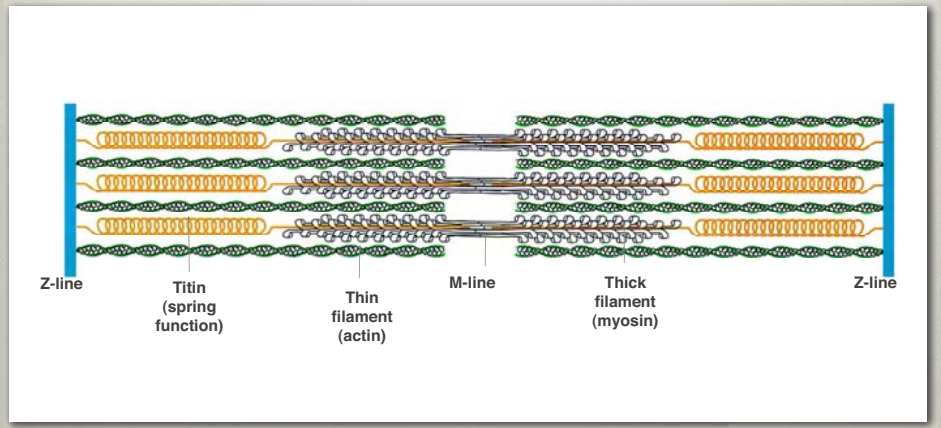
The myosin “cross-bridge” cycle



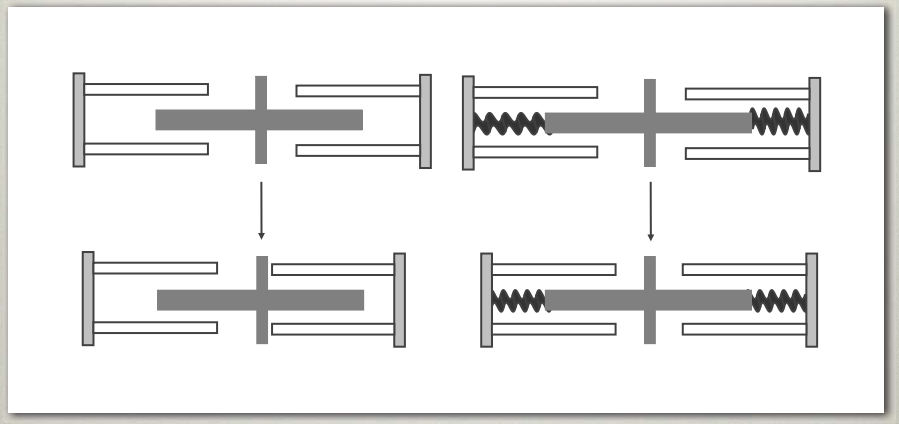




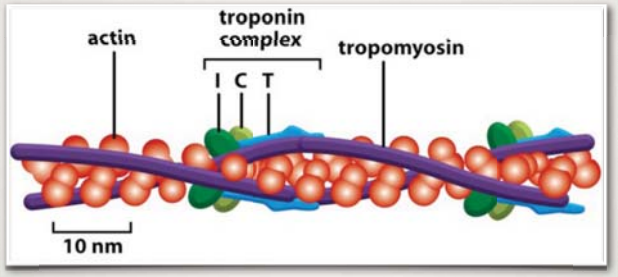
Elasticity of striated muscle

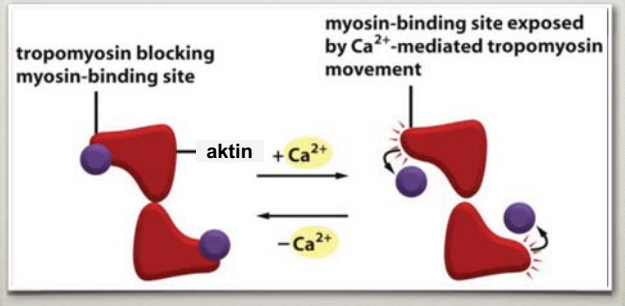


Role of titin in sarcomere: Limitation of A-band asymmetry



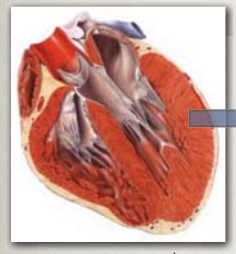
Contraction regulation in striated muscle

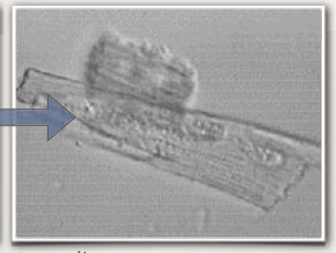




Biophysics of biological motion

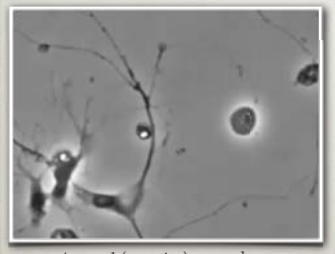
Types of biological motion



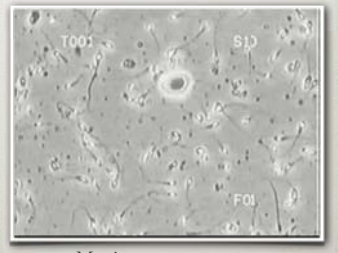


Autonomous cardiomyocyte

Dividing cell

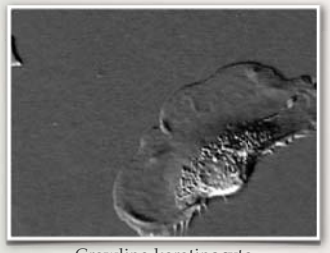


Axonal (neurite) growth



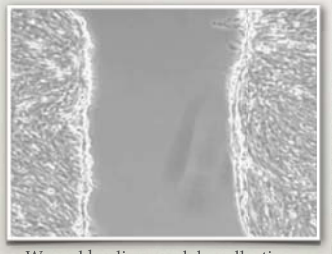
Moving spermatocytes

Types of biological motion

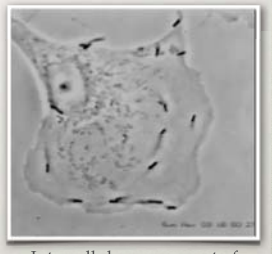


Crawling keratinocyte

Chemotaxis



Wound healing model - collective fibroblast moevent



Intracellular movement of pathogenic Listeria bacteria

The cytoskeletal system

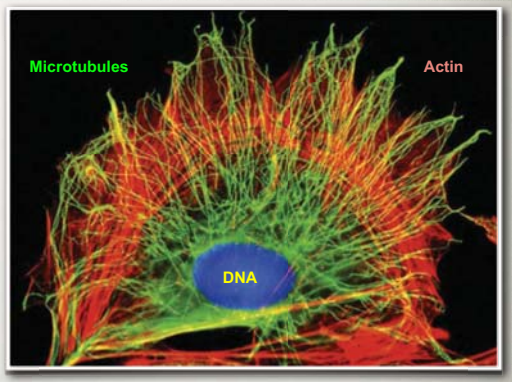
Dynamic filamentous system of eukaryotic cells

Three main filament classes:
- A. Thin (actin)
- B. Intermediate
- C. Microtubules

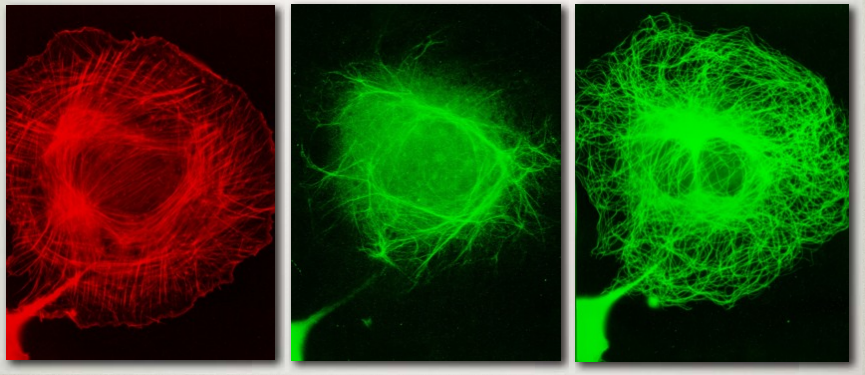
Filament mechanics is important

Polymerization: "smart brick" building blocks

Role:
- A. Movement, shape
- B. Cell division
- C. Intracellular transport



The cytoskeletal system



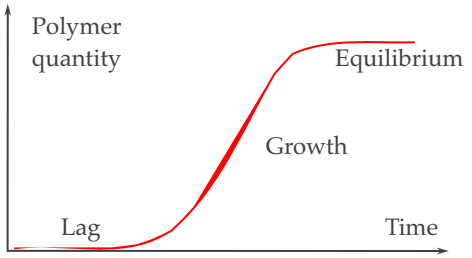
Actin (rodamin-phalloidin) | Vimentin (anti-vimentin) | Mikrotubules (GFP-tubulin)

1. Mechanics
2. Polymerization

Polymerization

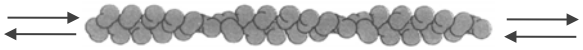
Process of the assembly of monomers

Phases of polymerization:
1. Lag phase: nucleation
2. Growth phase
3. Equilibrium phase

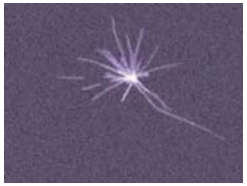


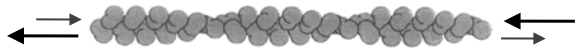
Polymerization equilibria

1. True equilibrium

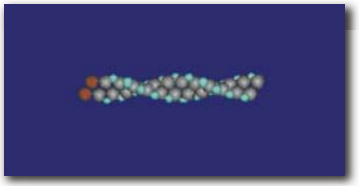


2. Dynamic instability: slow growth followed by "catastrophic" depolymerization

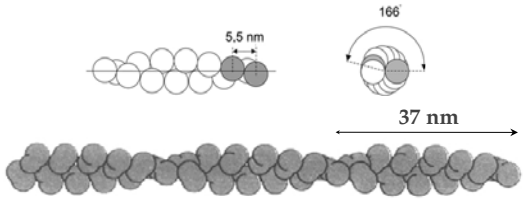


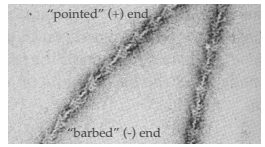


3. Treadmilling



The actin filament (F-actin)





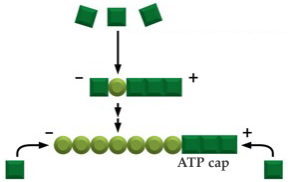
~7 nm thick, length *in vitro* exceeds 10 μm, *in vivo* 1-2 μm

Right-handed double helix.

Semiflexible polymer chain (persistence length: ~10 μm)

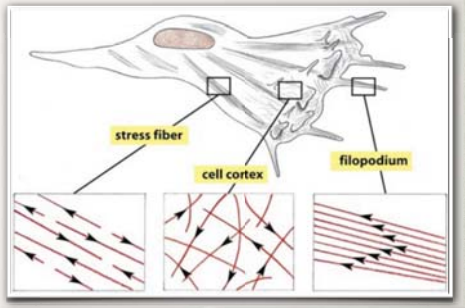
Structural polarity ("barbed", "pointed" ends)

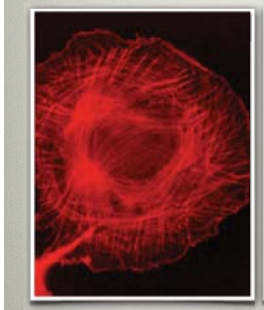
Asymmetric polymerization: ATP cap



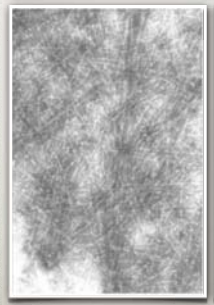
Actin in the cell

cortex
stress fibers,
cellular processes (lamellipodia, filopodia, microspikes, focal contacts, invagination)
microvillus

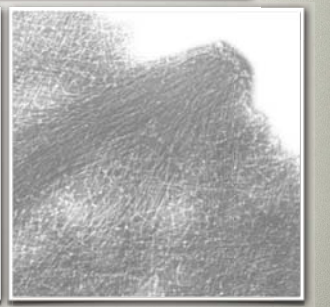




Stress fibers

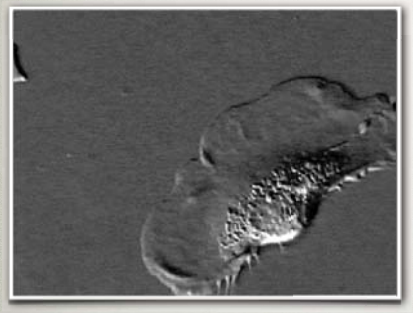


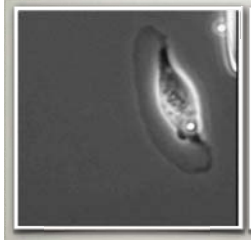
cortex

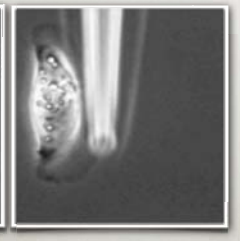


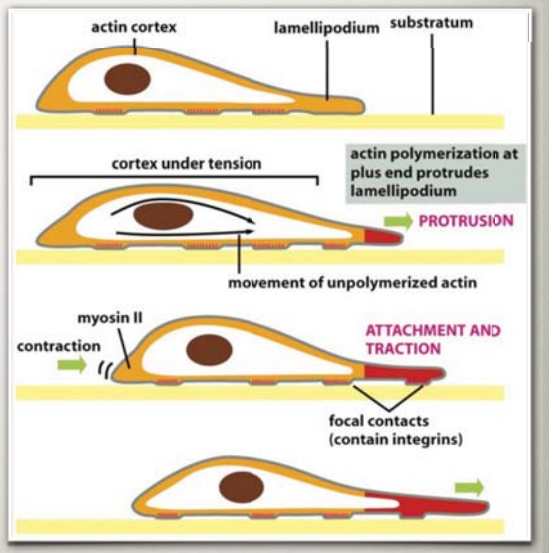
filopodium

Actin-dependent cell movement

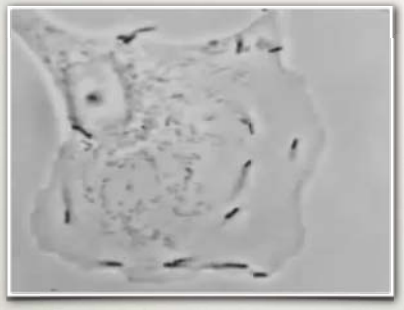




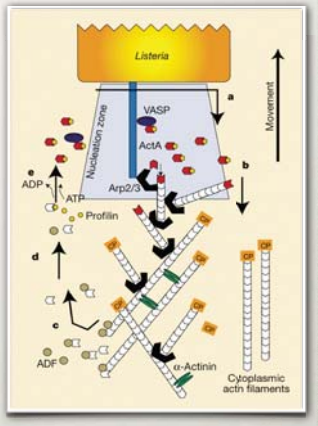




Intracellular pathogens make use of the actin system

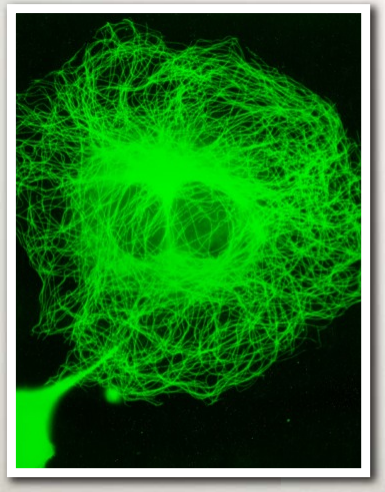


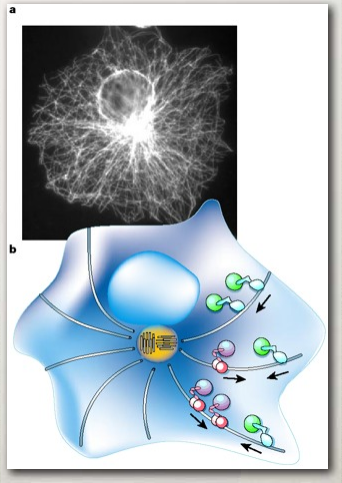
Intracellular motility of Listeria monocytogenes bacteria



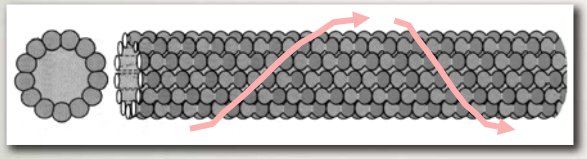
Microtubular system

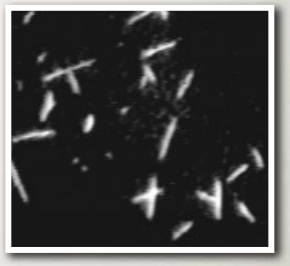
Filamentous system of eukaryotic cells composed of tubulin and its associated proteins





The microtubule

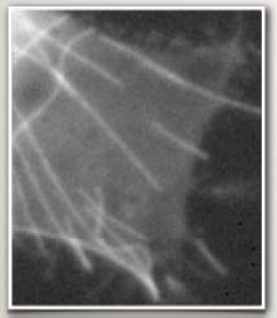




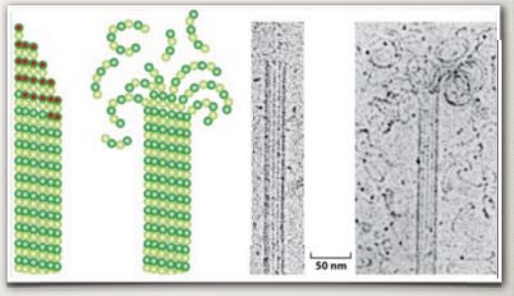
~25 nm in diameter, tubular structure
13 protofilaments
Right-handed short-pitch helix
Left-handed long-pitch helix
Rigid polymer chain (persistence or correlation length is a few mm!)
Structural polarity:
+end: rapid polymerization, terminated by β-subunit
-end: slow polymerization, terminated by α-subunit
GTP-cap

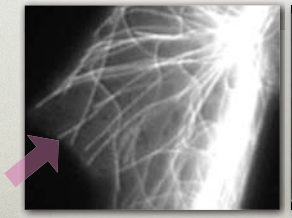
Polymerization equilibria in microtubules

Treadmilling



Dynamic instability



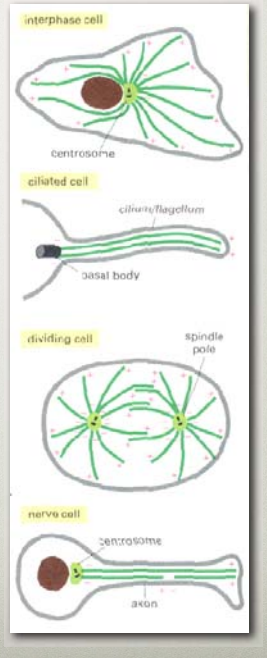


Microtubular system in the eukaryotic cell

Where?
Cytoplasm of interphase cell, axon, cilia, flagella, mitotic spindle.

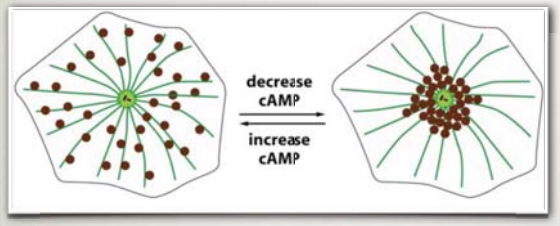
Polarity within the cell
-end in centrosome, +end in periphery.
Centrosome: 2 centrioles, centrosome matrix with γ-tubulin.

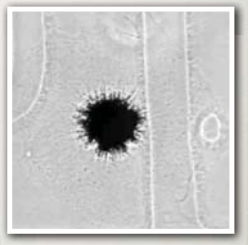
Microtubules might be involved in the commitment and fixation of cell polarity with the help of associated (capping) proteins.

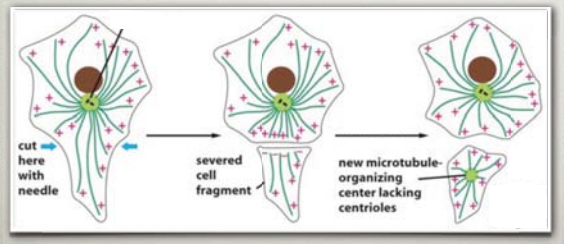


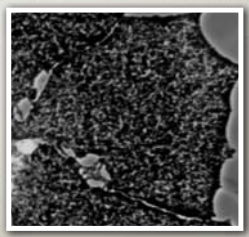
Functions of the microtubular system

1. "Highways" for motor proteins
2. Senses, monitors and finds the geometric center of the cell.
3. Motility functions (e.g., cell division)



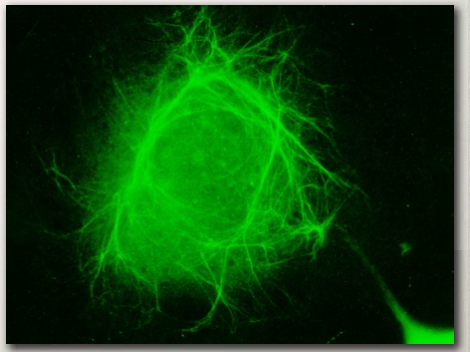






Intermediate filament system

Tissue-specific filamentous protein system composed of 8-10-nm filaments, found on most animal cell types.
Fundamental biological function is providing mechanical stability.



Vimentin, Vic Small

Intermediate filament building blocks

Intermediate filament dimer:

Properties:

-Chemically resistant (detergents, high ionic strength)
-Can be extracted with denaturants (e.g., urea)
-Fibrous monomer (not globular as actin or tubulin)
-amino-terminal head
    -central rod (α-helix, heptad repeat)
-carboxy-terminal tail
    -tissue-specific monomers differ in their terminal sequences

Classification of intermediate filaments

Based on tissue specificity
(Classical categories)

Tissue type	Intermediate filament
Epithelium	Keratins
Muscle	Desmin
Mesenchyme	Vimentin
Glia	Glial fibrillar acidic protein (GFAP)
Nerve	Neurofilaments (NF-L, NF-M, NF-H)

Polymerization of intermediate filaments

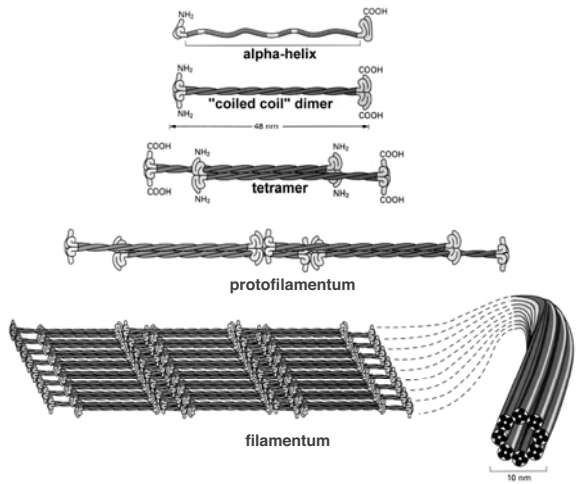
Fully polymerized state in the cell (not dynamic equilibrium)

Central rods (α-helix) hydrophobic interactions -> colied-coil dimer

2 dimers -> tetramer (antiparallel arrangement, structural apolarity)

Longitudinal association of tetramers -> protofilament

8 protofilaments -> filament

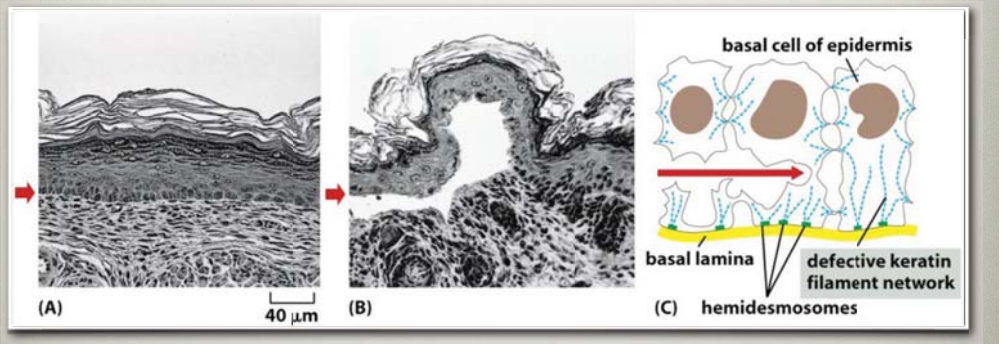


Tissue functions of intemediate filaments

Providing mechanical stability

Epithelial cells:

-Pathology: *epidermolysis bullosa simplex*. Mutation in the keratin gene.
Bullous epithelial destruction upon minor mechanical effects.



MOTOR PROTEINS

1. Bind to specific filaments
2. Generate force and displacement
3. Convert chemical energy to mechanical

Types of motor proteins

1. Actin based
Myosins: Conventional (myosin II) and non-conventional
Myosin superfamily (I-XXIV classes). Move towards plus end.

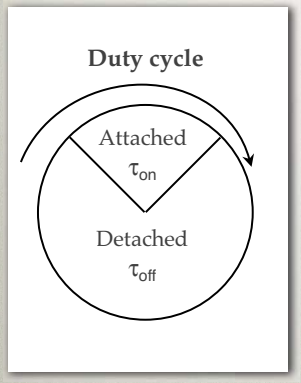
2. Microtubule based
a. Dyneins: Ciliary (flagellar) and cytoplasmic dyneins.
Move towards the minus end along the microtubule.
b. Kinesins: Kinesin superfamily: conventional and non-conventional.
Move towards the plus end along the microtubule.
c. Dynamins: MT-dependent GTPase activity
Biological role: vacuolar protein sorting (pinchase enzymes)?

3. DNA based motors
DNA and RNA polymerases, virus capsid packaging motor, condensins
Produce force and displacement along the DNA strand

4. Rotary motors
F1F0-ATP synthase
Bacterial flagellar motor

5. Mechanoenzyme complexes
Ribosome

Duty cycle of motor proteins



"Duty ratio": $r = \frac{\delta V}{v}$

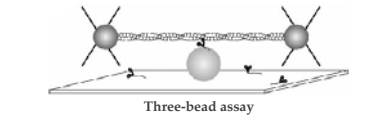
δ=working distance
V=ATPase rate
v=sliding velocity

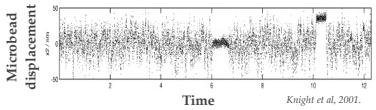
Processive motor: r->1
E.g., kinesin, DNA-, RNA-polymerase.
Remains attached throughout most of the duty cycle.
Carries its load by itself.

Non-processive motor: r->0
E.g., myosin.
Remains detached throughout most of the duty cycle.
Works in ensembles.

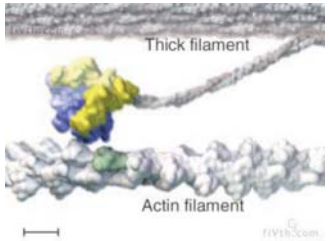
Force generated by a single motor protein: **few pN**.

Non-processive motor proteins
Myosin


Three-bead assay

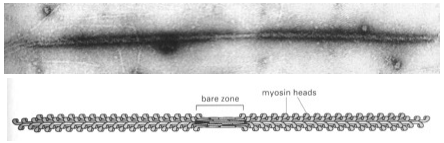


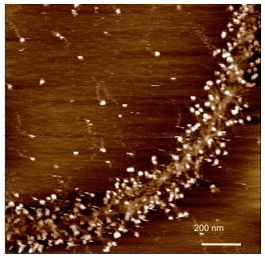
Knight et al, 2001.



Step size: **5.5 nm**
(distance between neighboring actin subunits)

Non-processive motors work in ensembles



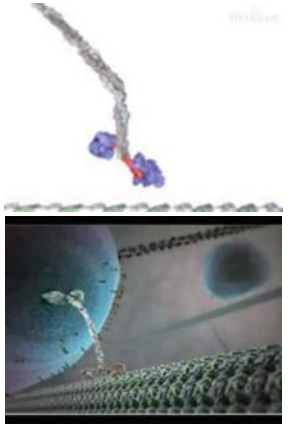


Synthetic thick filament
AFM image

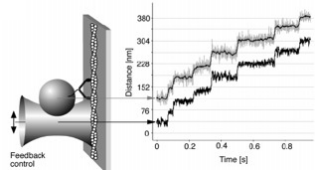
PROCESSIVE MOTOR PROTEINS

Kinesin

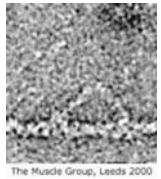
Step size: **8 nm**
(distance between every other tubulin subunit)



Myosin V

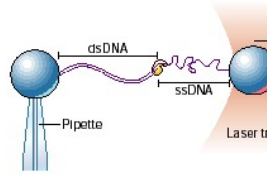


Step size: **~36 nm**
(half pitch along actin helix)

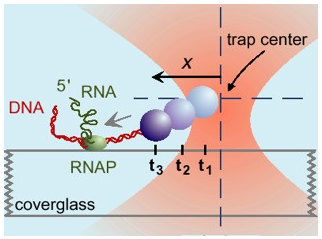


Processive motors work alone.

DNA Motors

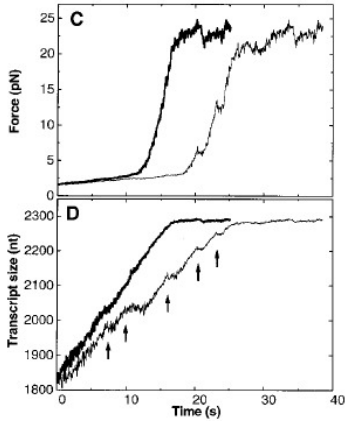


T7 DNA Polymerase



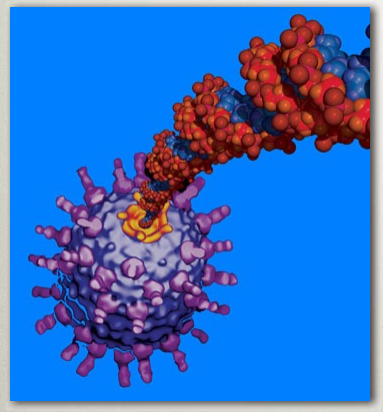
RNA Polymerase

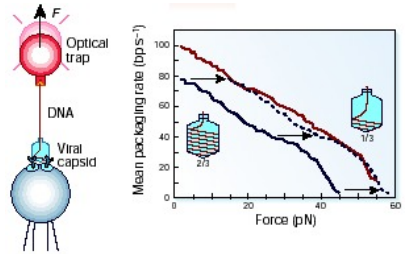
Processive motors



RNA Polymerase , Wang et al. 1998.

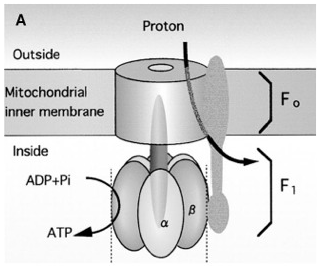
Virus portal motor
Special DNA motor

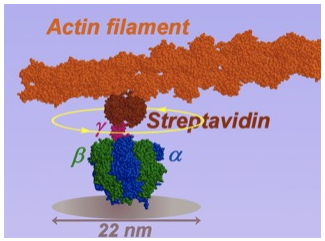


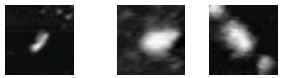


ϕ29 bacteriophage portal motor

ROTARY MOTORS I:
F1F0-ATP Synthase

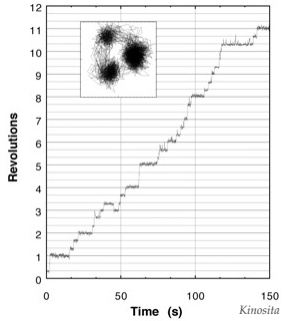






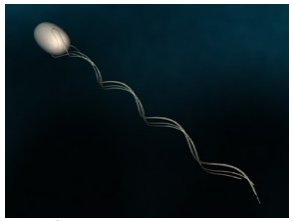
20 nM ATP 200 nM ATP

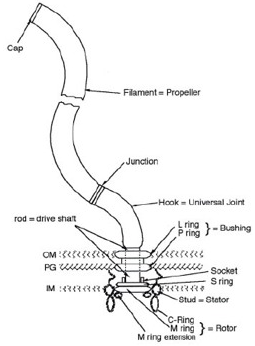
Discrete 120° rotational steps

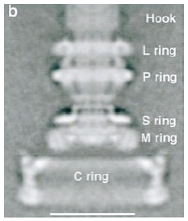


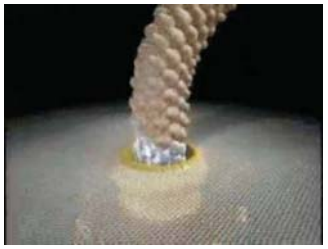
Kinosita

ROTARY MOTORS II:
Bacterial flagellar motor









Speed: > 20000 rpm
Energy consumption: 10^{-16} W
Efficiency: > 80%
Energy source: protons