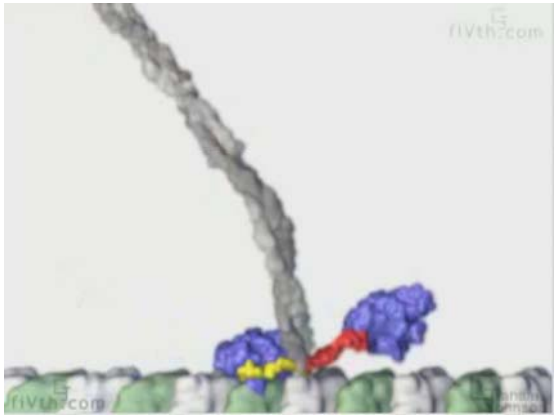
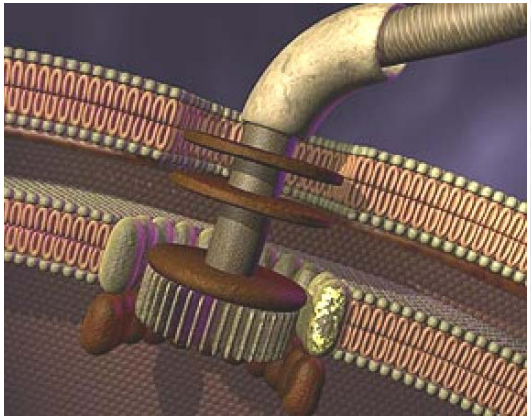
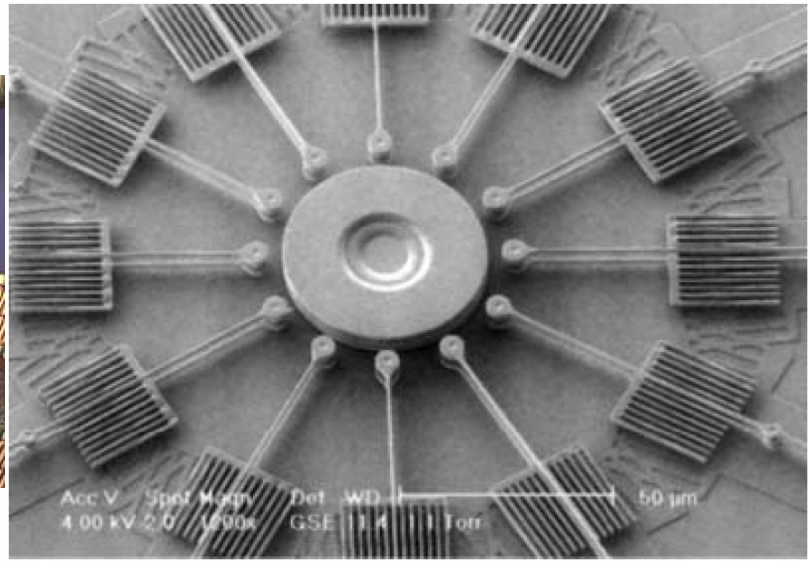
Biological Machines, Cell Mechanics and Nanotechnology







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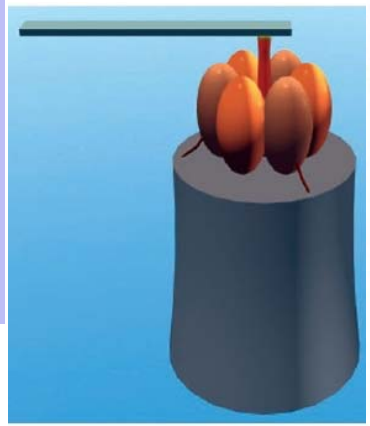
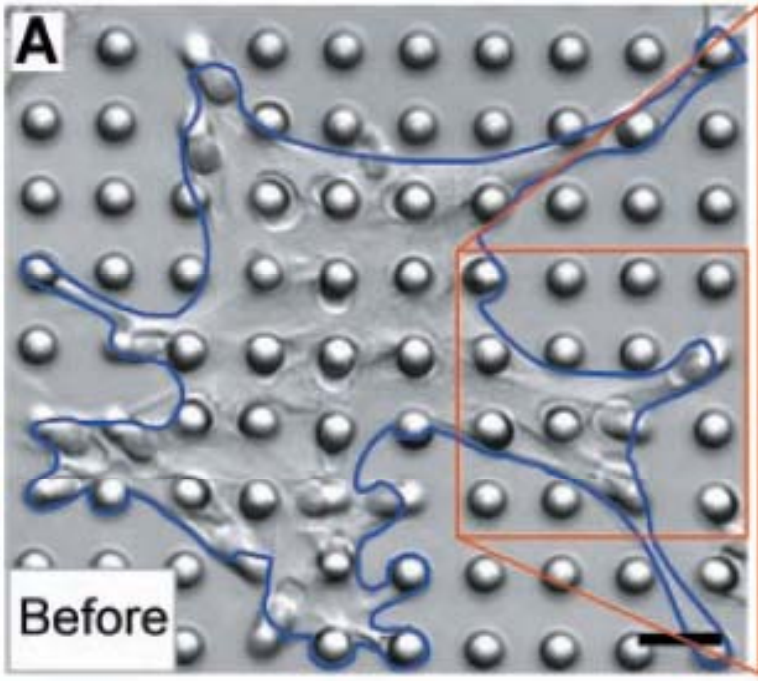
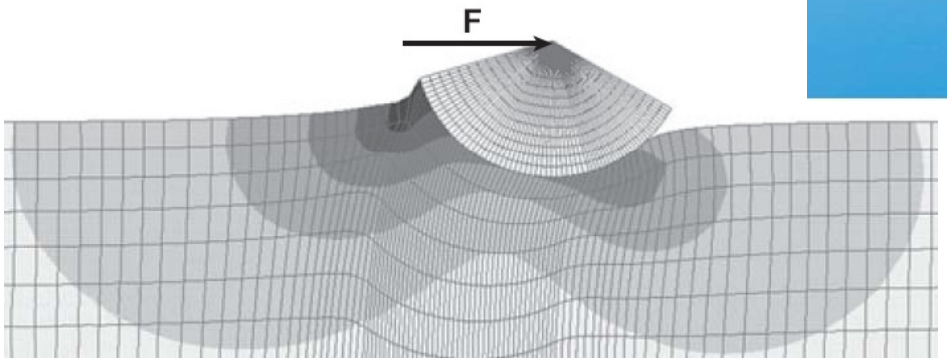






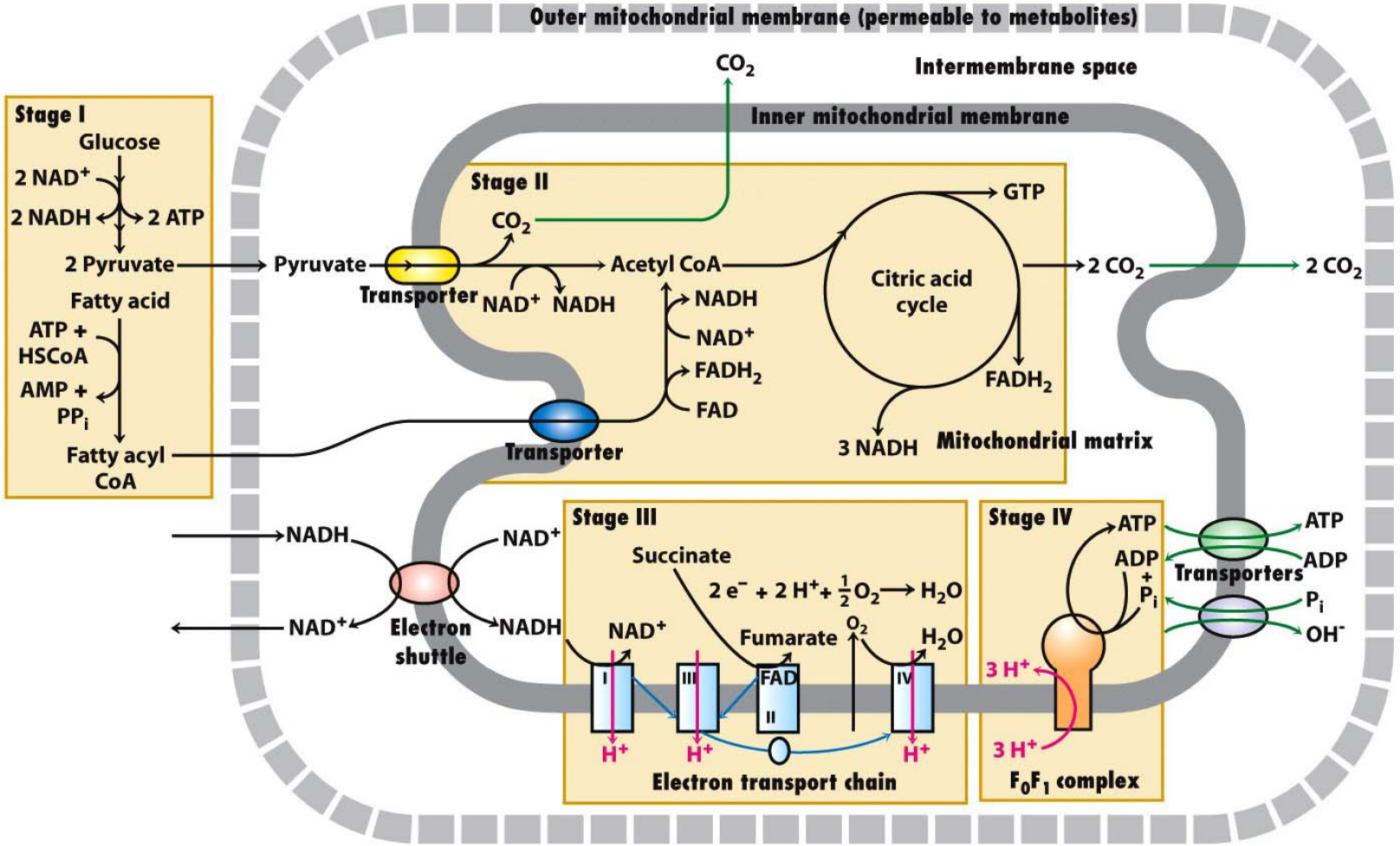
What is the simplest molecular machine?

Besides sophisticated ATPase and complex ion channels, there is a very simple “machine” in the mitochondria: the **Citric Acid Cycle (CAC)**

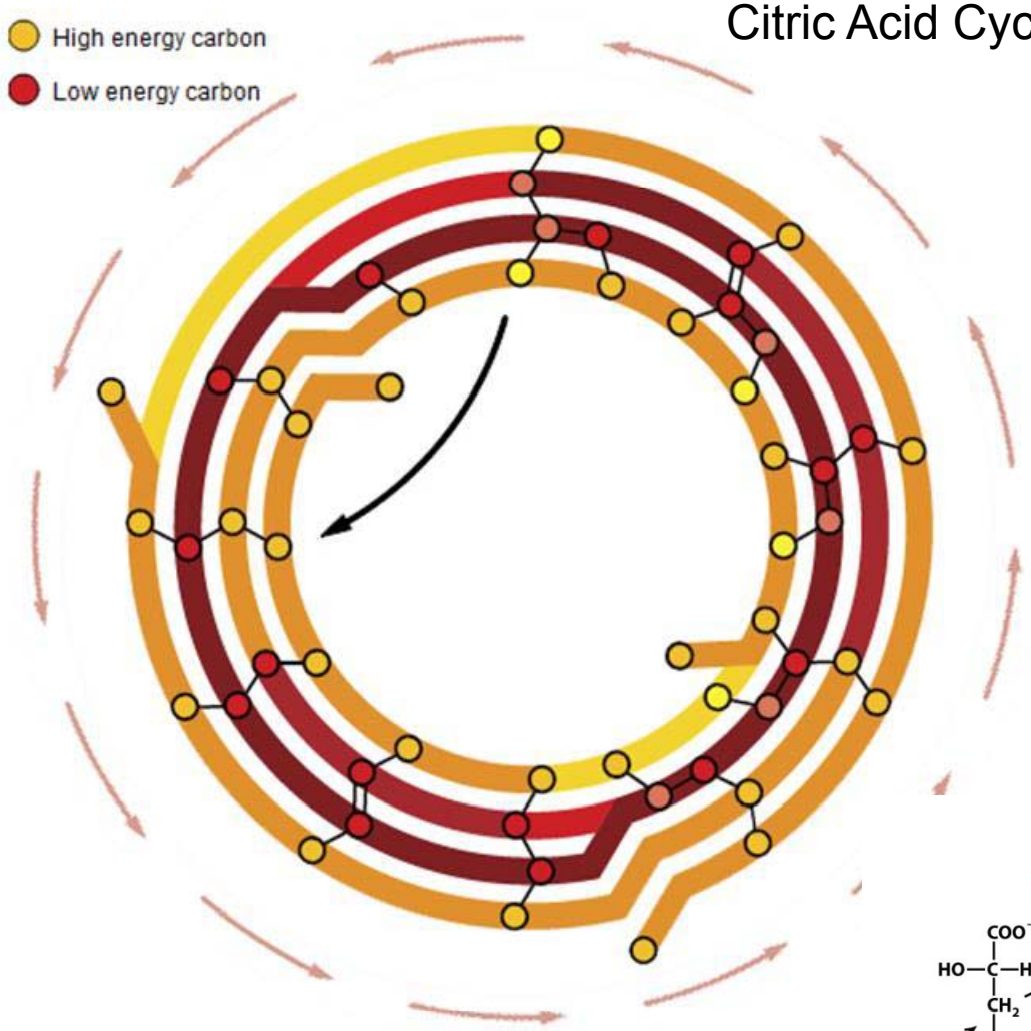
⇒ Is the CAC the most simplest and maybe the ancestor of all “biological machines”?

⇒ Since it is based on **pure chemical reactions** (chemistry evolved long before “biology”)

⇒ These **“geochemical” reactions** can occur even in very unfavorable conditions (unfavorable environments other as those on earth)

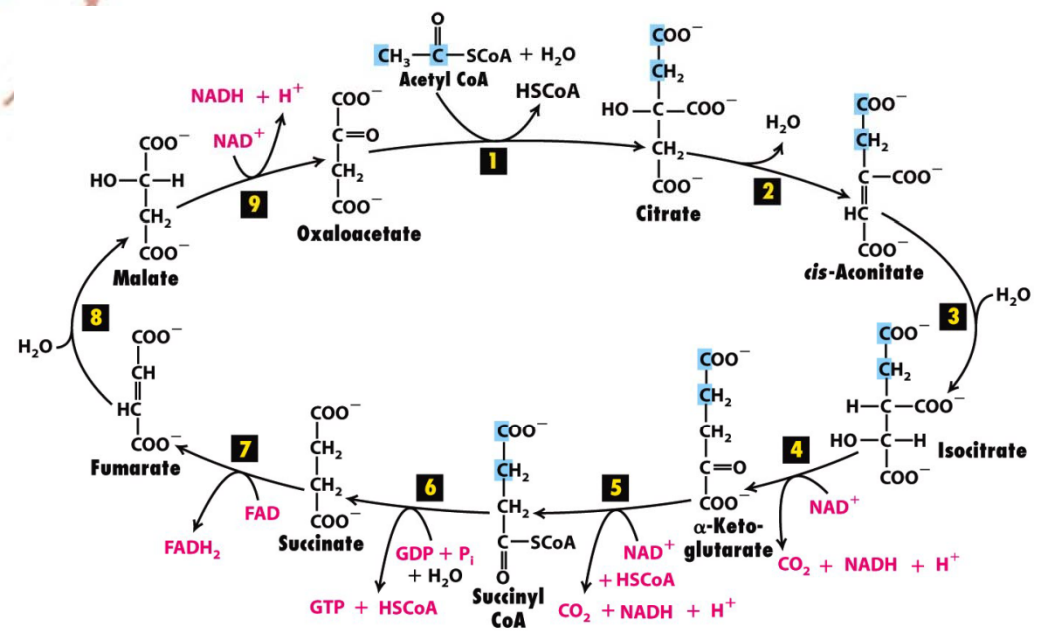


Citric Acid Cycle: the most ancient biological machine?



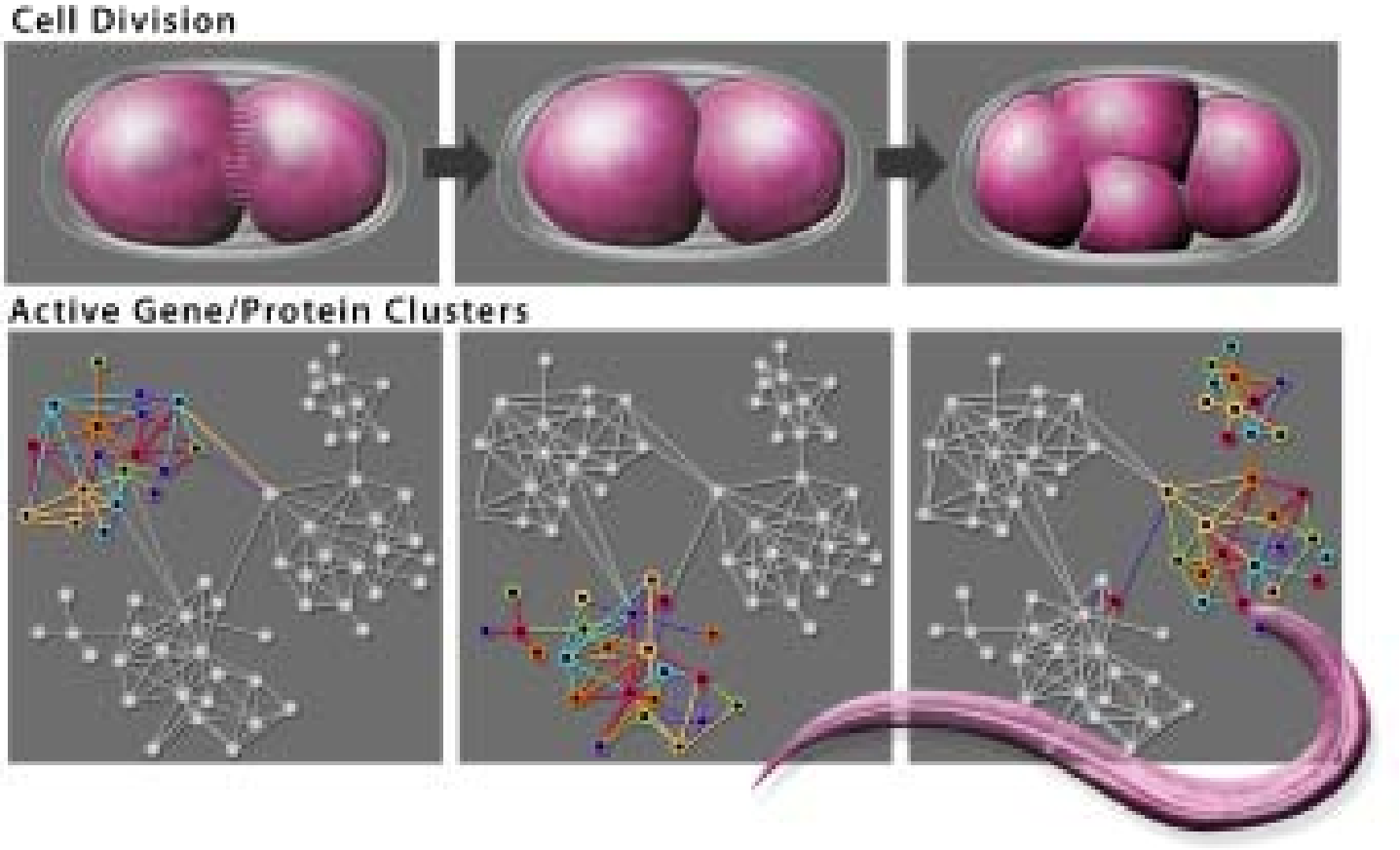
In the CAC, the energetic molecule **Acetyl-CoA** (two carbon atoms) is **metabolized in** $\underline{CO_2}$ and high-energy electron carriers ($\underline{NADH}$ and $\underline{FADH_2}$).

The reductive citric acid cycle **behaves like a chemical hurricane**. [Carbon atoms from CO_2 (yellow and orange) attach at either end of molecules. As the cycle proceeds (counter-clockwise), they are drawn toward the interior (red) until the molecule splits at the top of the cycle, creating two smaller molecules (curved arrow), which then repeat the cycle.]



Protein clusters as macro-biological molecular machines?

Genomics, proteomics and bioinformatics were used to identify active and non-active gene- and **protein clusters** during the development of *C. elegans* embryos



Discrete and interconnected gene- and protein clusters are turned on and off during different developmental stages

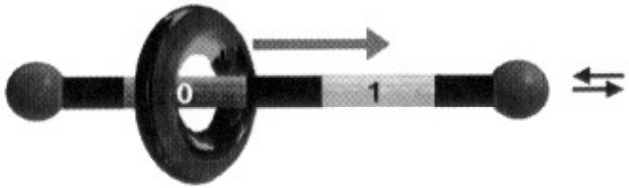
Synthetic molecular motors

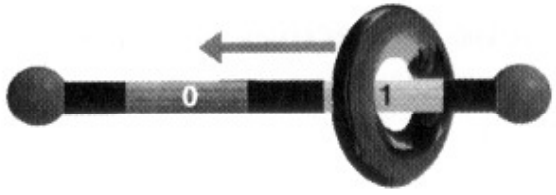
Biological motors offer great inspiration for the design of **artificial motors** to achieve controlled movement at the molecular level

Rotaxane systems:

• A **macrocylce** (*train*) can travel along a **molecular chain** (*rail*) between two *stations* (0,1)

• *Train's* position depend on the electron composition of the *stations* and *train*





• Translocation of train can be achieved by redox or acid/base stimuli as well as photochemically

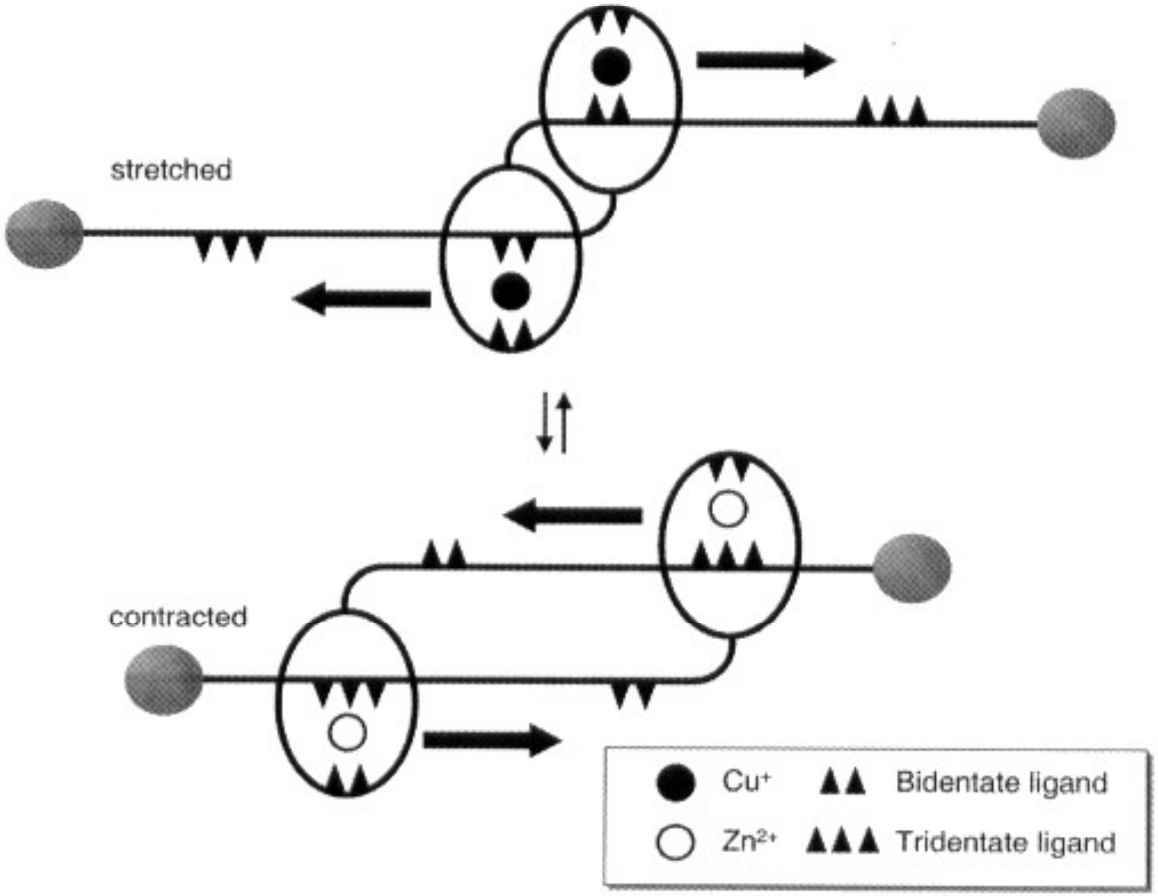
• Translocation is initiated by **protonation of station 0** making the interaction between **train and station repulsive** (train moves to station 1 as a result)

• After deprotonation the system relax back to its initial state (train back to station 0)

Schliwa, Molecular Motors, 1st Ed.

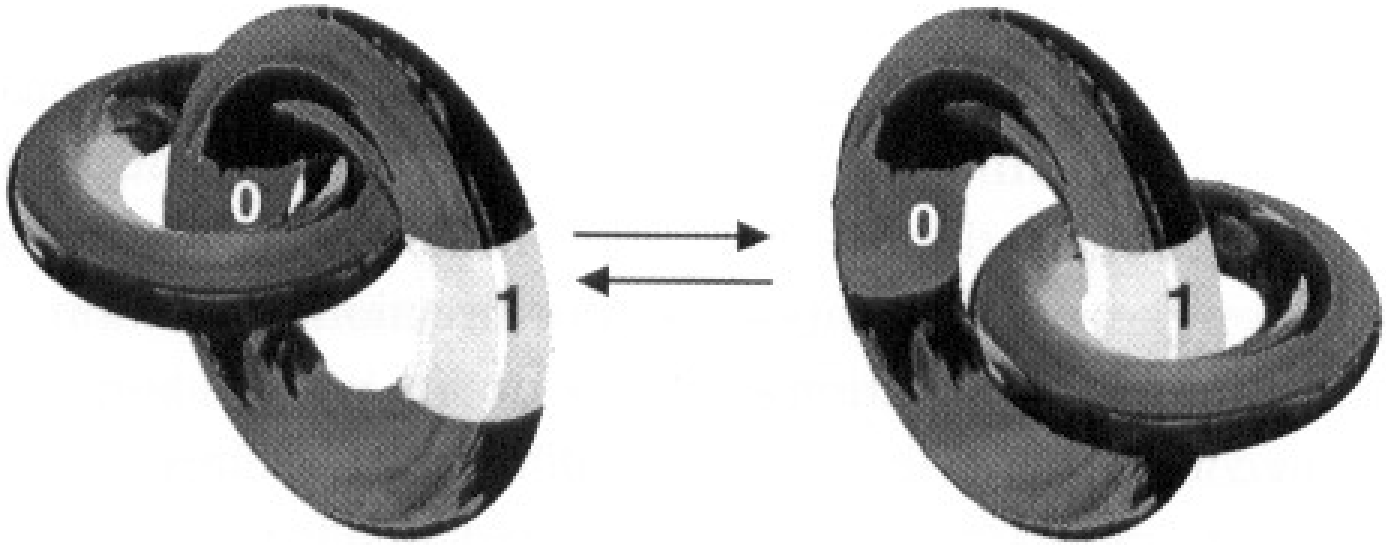
Synthetic molecular muscle

- Two linear **intertwined rotaxane** units can <u>contract and stretch like a muscle</u>
- In the presence of Cu^{+} the conformation is **stretched**
- In the presence of Zn^{2+} the configuration is **contracted**



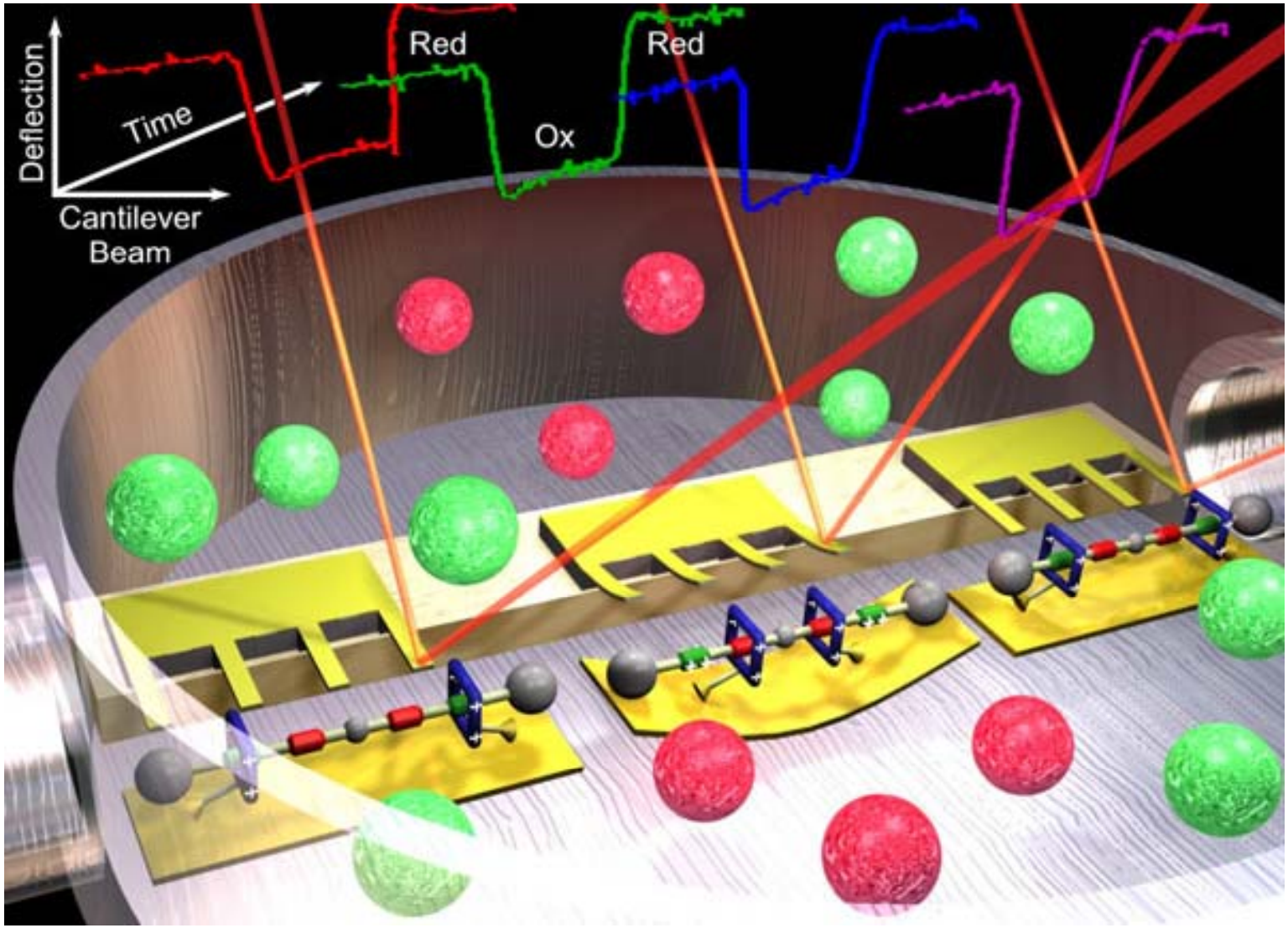
Synthetic molecular rotary motors: Catenanes

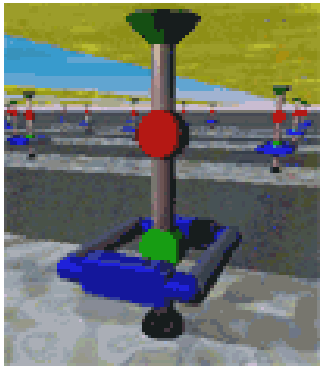
- Catenanes closely **resemble rotaxanes** but consisting of two interlocked rings
- One ring is analogous to the *train* and the other ring can be considered as the *rail*
- **Problem**: no unidirectional rotation => statistically only 50% full rotations possible
- However, the problem of unidirectional rotary motion as been solved (not shown)



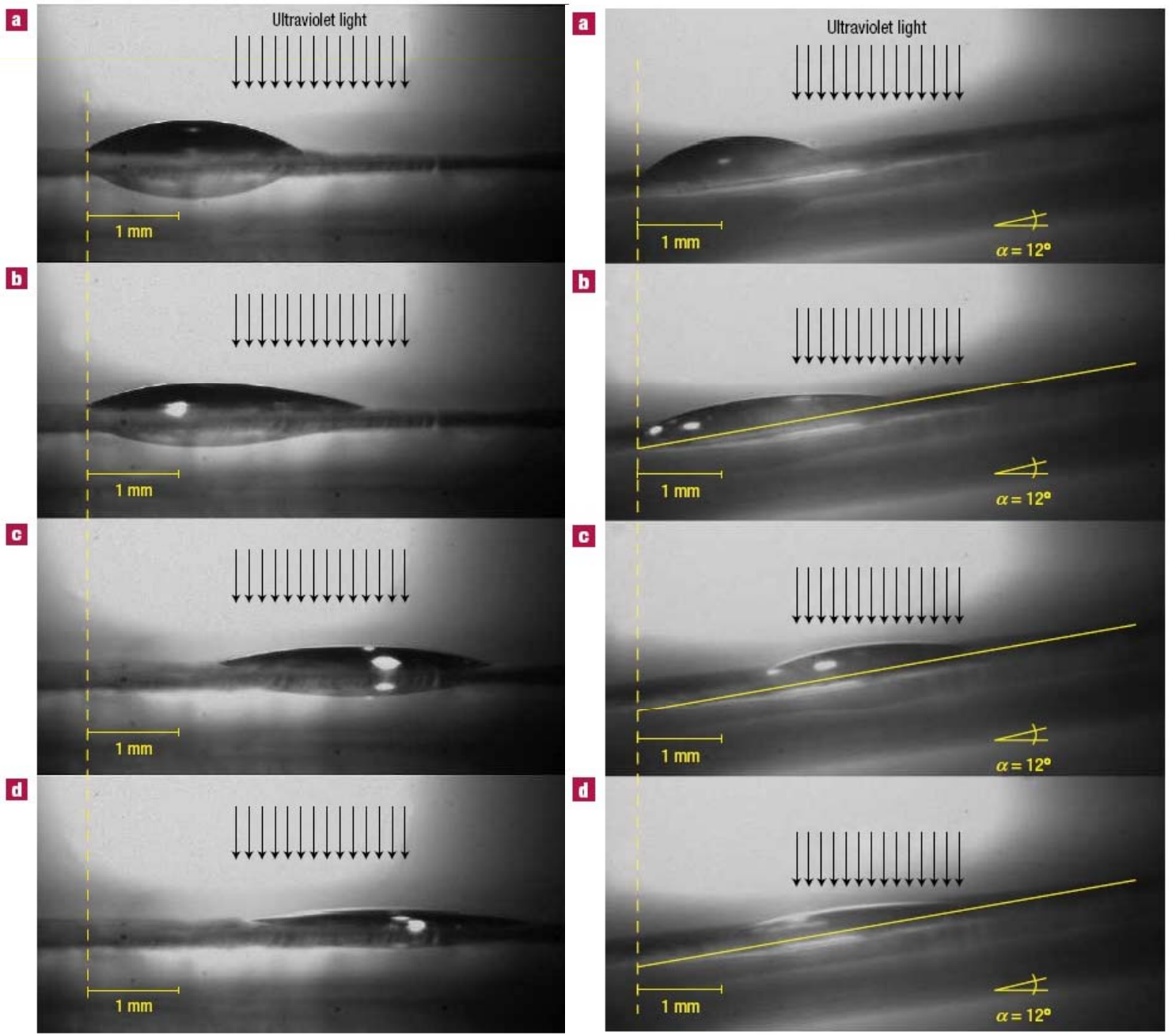
Molecular muscles

NEMS (Nanoelectromechanical systems) device based on rotaxane coated AFM cantilevers: redox-driven contraction/relaxation of rotaxanes results in a measureable deflection of the laserbeam





“Magic” movement of an liquid drop by rotaxanes



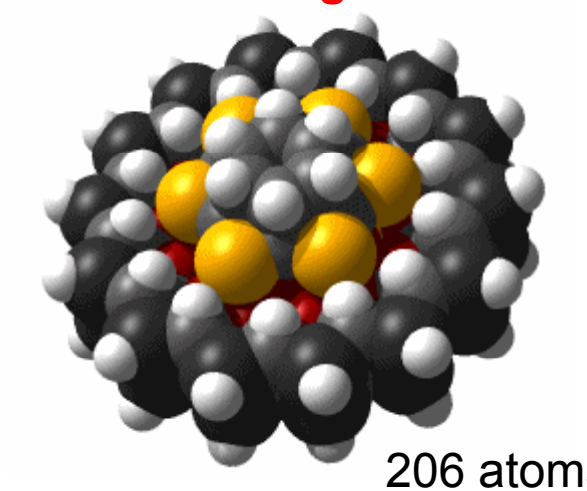
A monolayer of rotaxanes (turned on and off by UV-light) was able to move an liquid drop (1.25 µl) on a steep surface

Berná et al., Nat. Mater., 2005

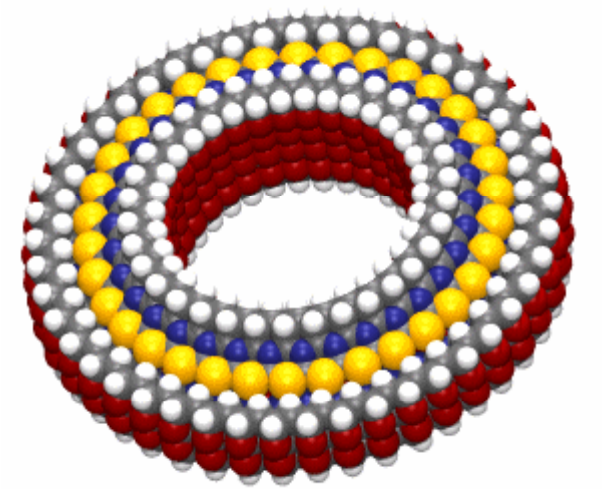
Computer models of non-biological nano-machines

- Many **macroscopic machines** can be reduced to the nano-level
- Some might work even better (no friction, no wearing/tiring) some might be impossible to design based on their complexity (e.g., atomic power plant)
- Examples of current modeled nano-constructions are:
  - Nano Bearing
  - Nano Gear
  - Nano Filter
  - Nano Pump
  - Nano Electromotor/ Nano Car
  - Nano Computer (simple I/O)
- A nano-bearing does not need any bearingballs or lubricants
- It works based on **strong** covalent bonds and **weak** "van der Waals" repulsive forces
- **Simulations** are based on reliable software tools already used by Chemists for many years

Nano-bearings



206 atoms



2,808 atoms

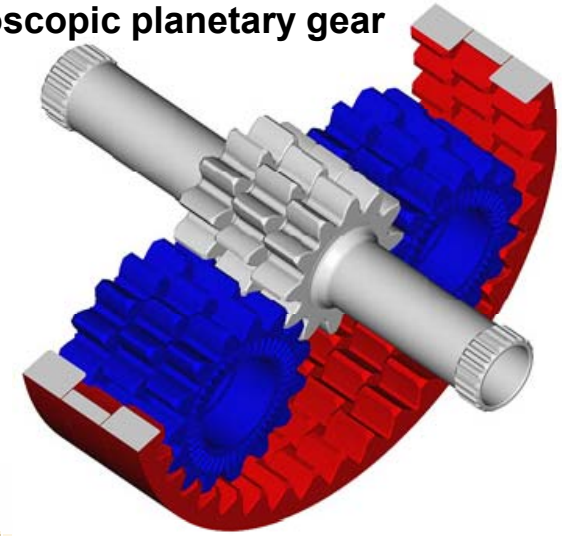
Macroscopic bearing with bearing balls embedded in lubricant

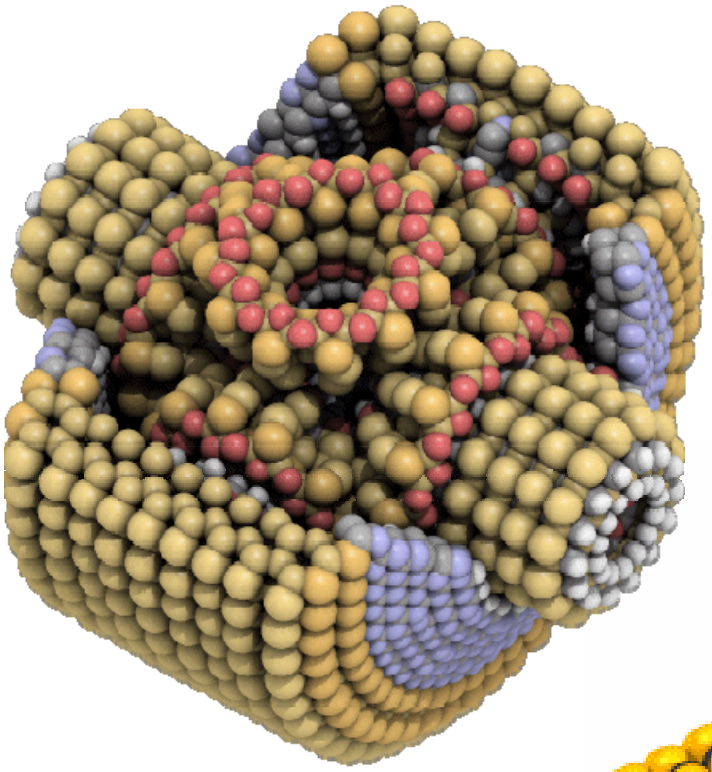
http://www.e-drexler.com/

Computer models of non-biological nano-machines

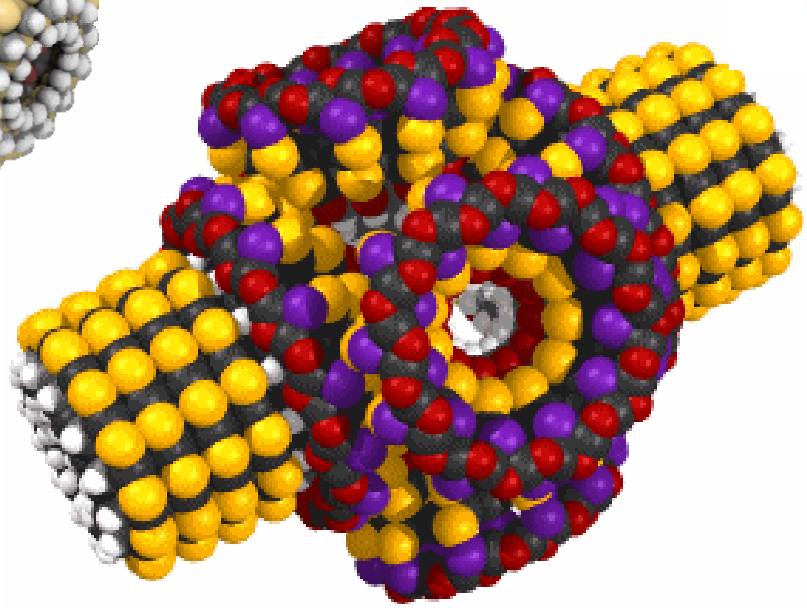
- Planetary gearing is a gear system that consists of one or more outer gears, or **planet gears**, revolving about a central, or **sun gear**
- Planetary gears **convert shaft power** from one angular frequency to another

Macroscopic planetary gear





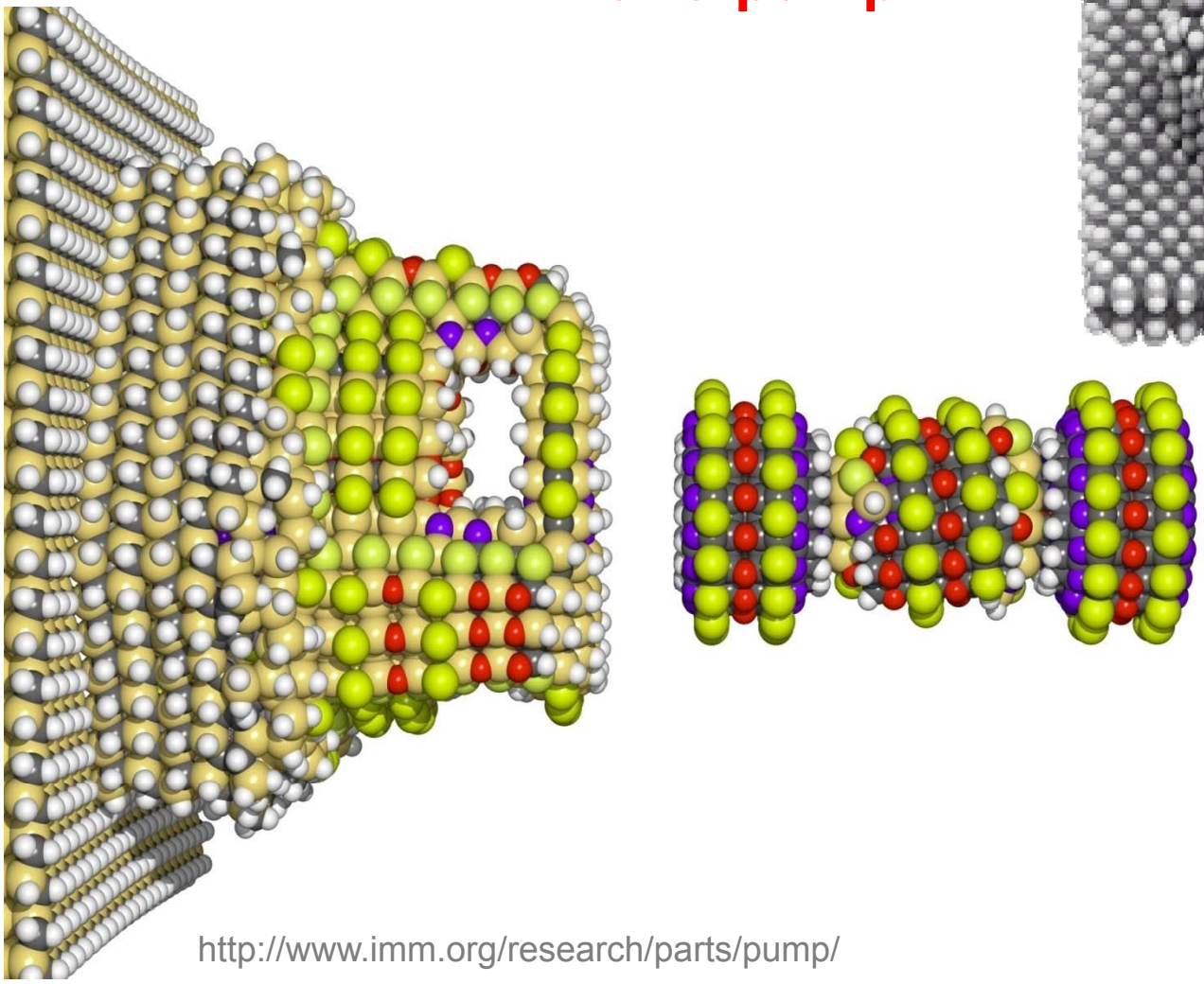
Nano planetary gear

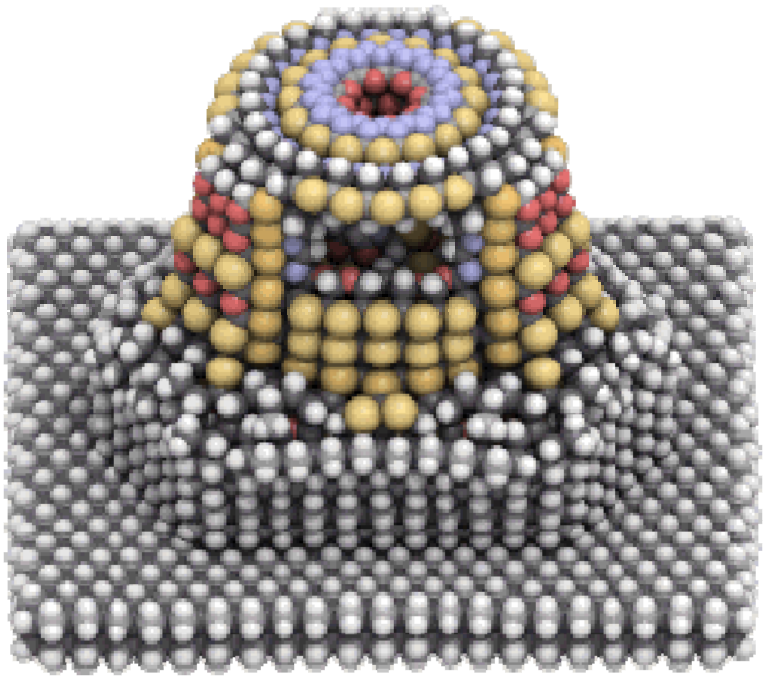


http://nanoengineer-1.com/

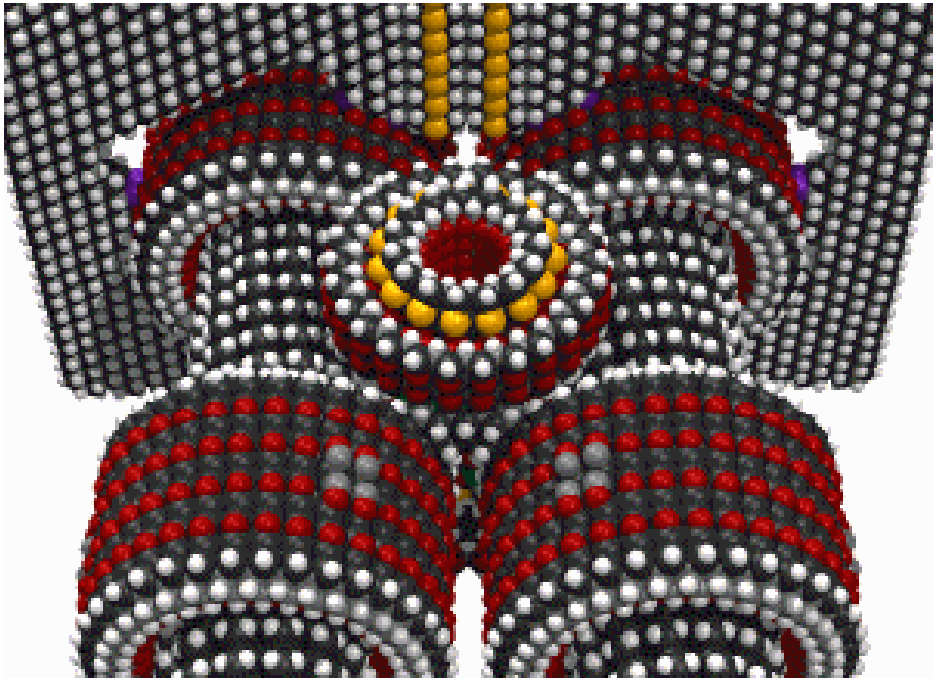
Computer models of non-biological nano-machines

Nano-pump



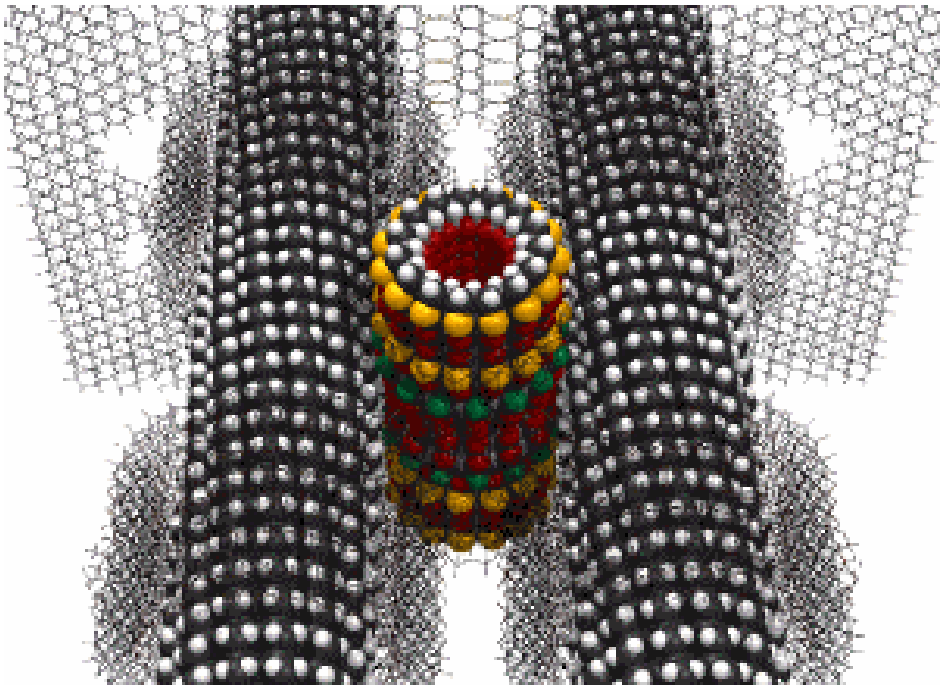


http://www.imm.org/research/parts/pump/

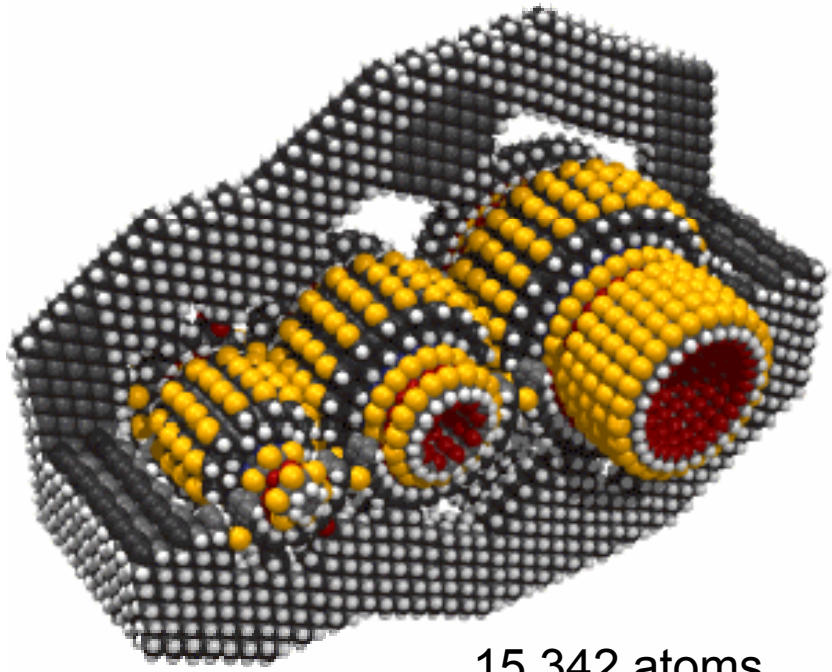


Complex nano-machines

- **Nano-worm drive** assembly containing 11 components made from 25,374 atoms
- Simulations **took 340 hours** to complete (on a regular desk-top computer)



Nano speed gear reducer

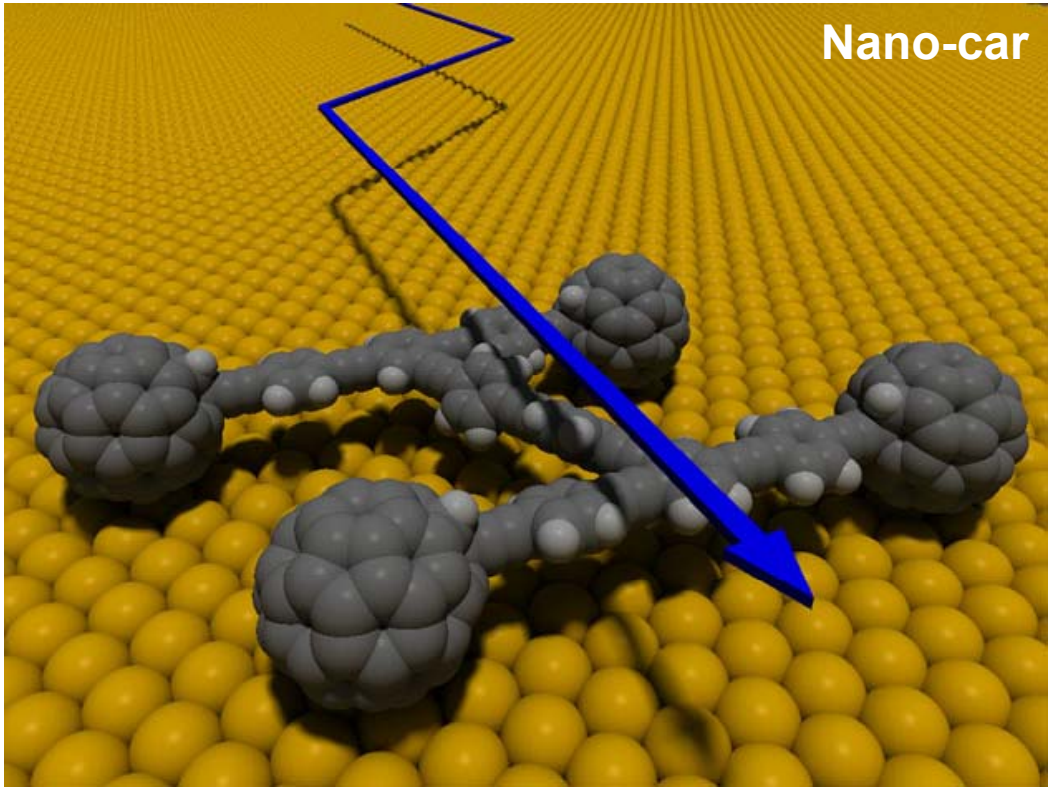


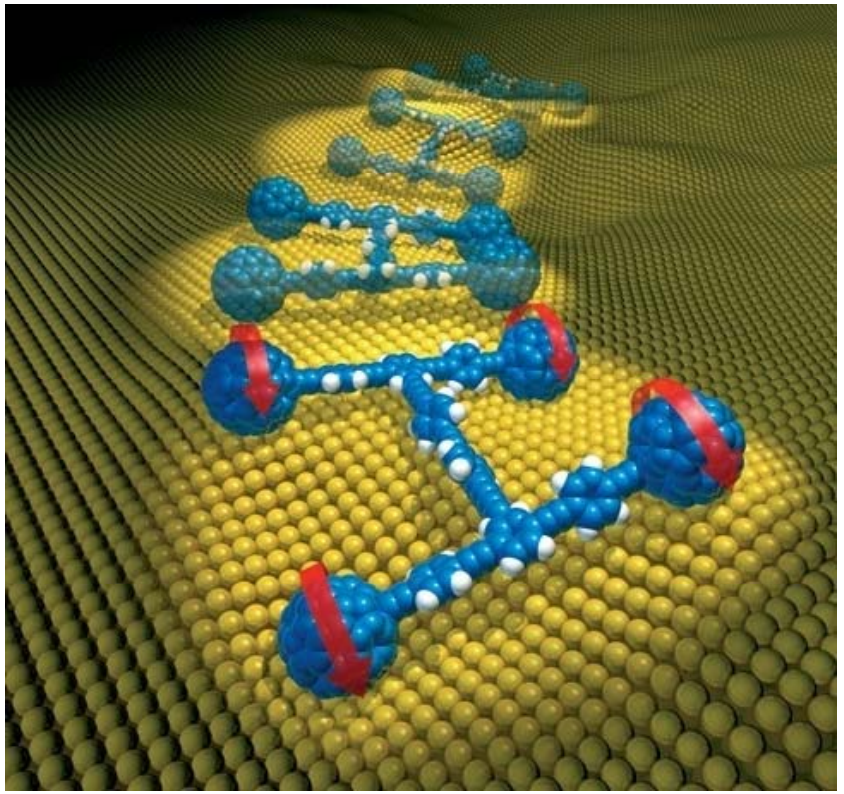
15,342 atoms

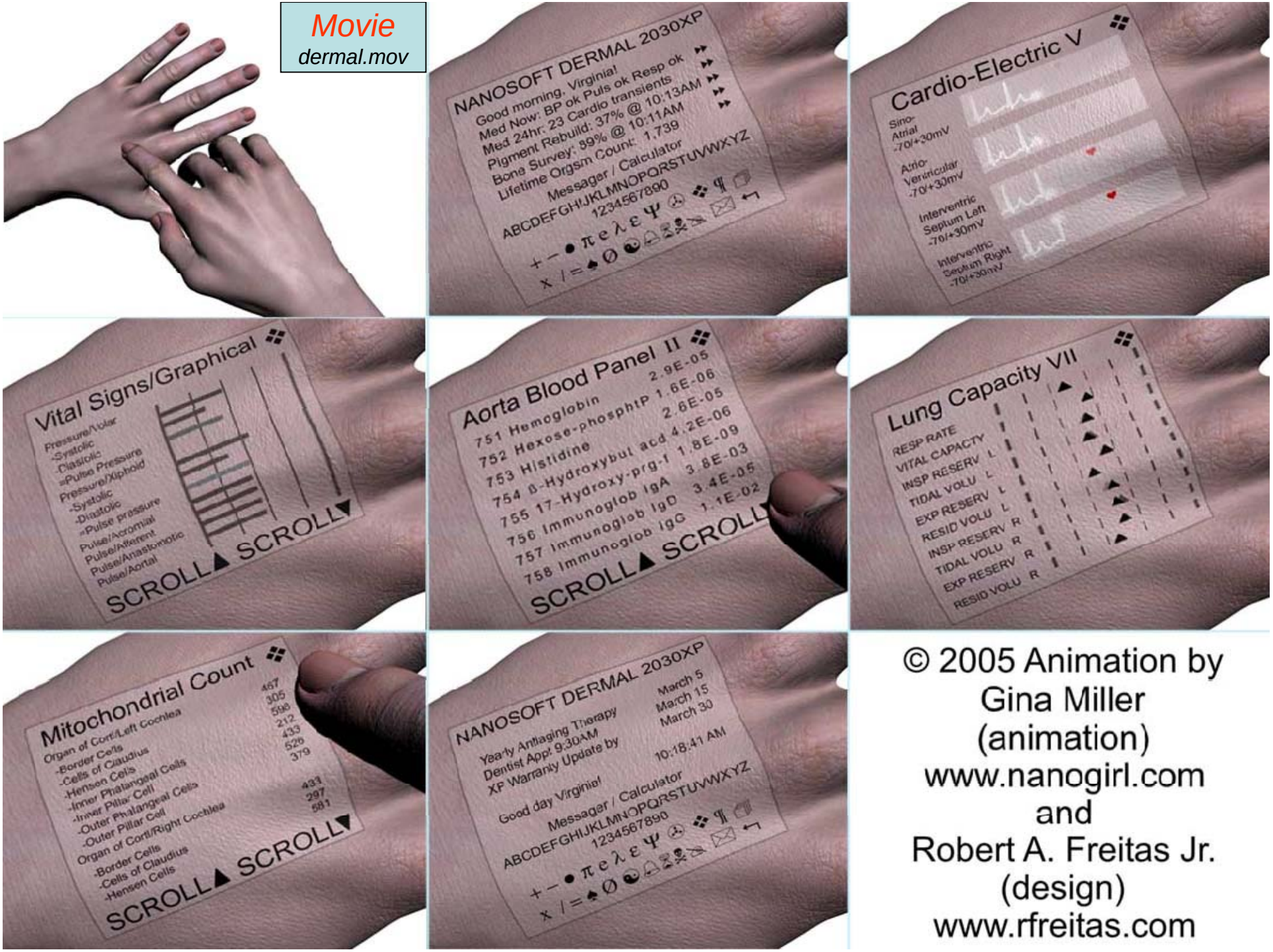
http://www.e-drexler.com/

Questions, applications critique

• It's **only a matter of time** before nanotechnology (combined with MEMS and optofluidics) is applied to the development of **neuroprosthetic devices**, **artificial retina** etc.
• Very far from now perhaps a brain implant using biological molecules to store data can back-up human memories (which might otherwise be lost due to degenerative diseases)
• It might be feasible to think of atom-by-atom manufacturing of such components in **nanofactories**
• However: The two machines containing about 25,000 atoms, are the most complex simulations ever created and they haven't even been built yet!
• By comparison: An ion channel (one of nature's sophisticated nanomachines) can have a molecular mass approaching 1,000 kD, and contains **millions of atoms**

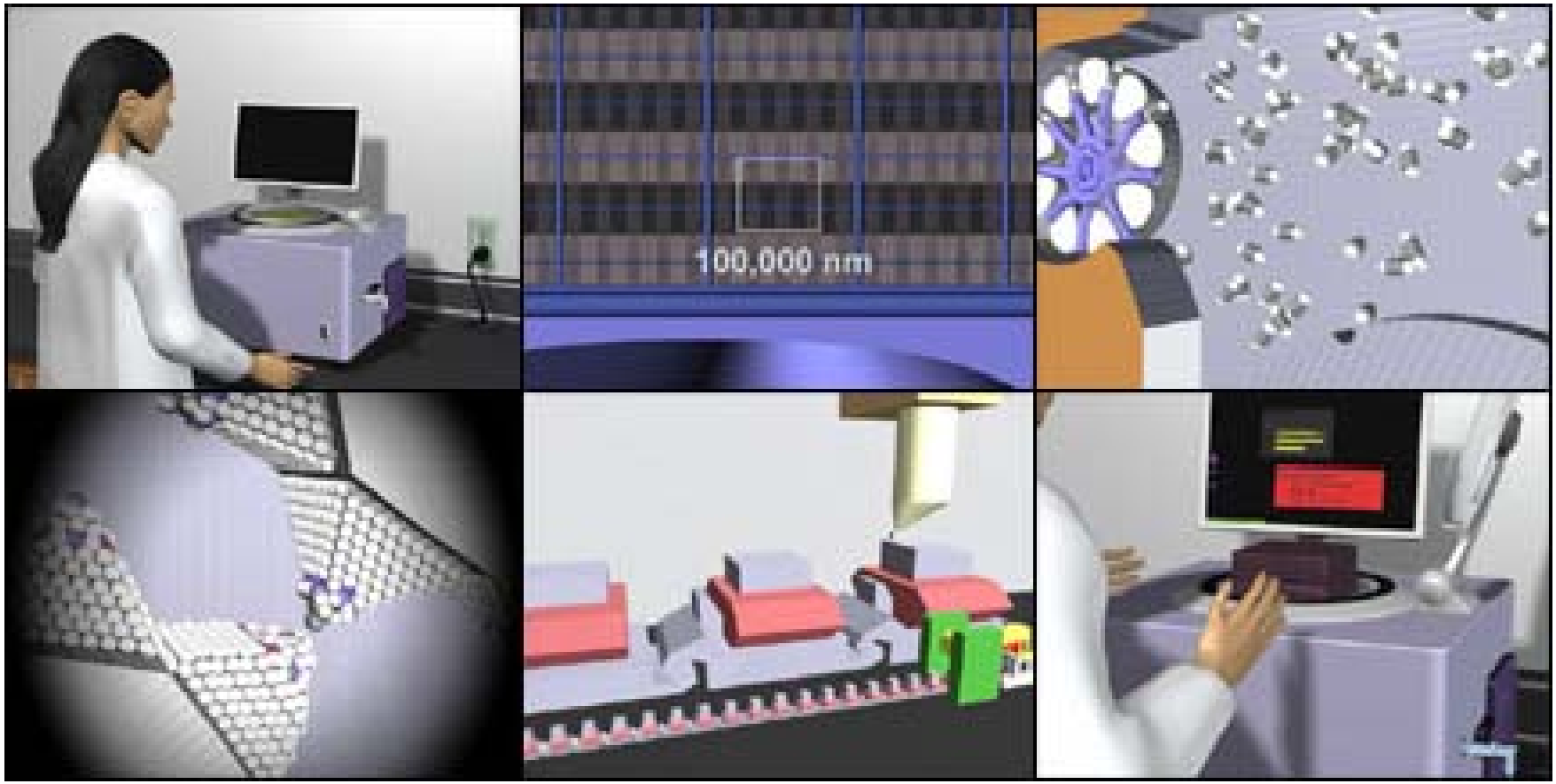






Nano Factory

Movie
NanoFactory.mov



http://www.e-drexler.com/

What is nature, what is life, what is a machine?

• Since **we are composed of units** that can be dissect into parts, modules, domains, proteins and atoms the question might arose: **Is life artificial?**
• Protein motors, intercellular sensors, membrane channels, protein scaffolds etc. leads to an **mechanistic** **understanding of the cell** (contrary to **vitalist** view)
• However: **Less fruitful** doing biological research is to pull organisms apart and inspecting them piece by piece (**reductionism**)

• A distinction between natural and artificial goes back at least to **Aristotle** and **Plato** but this distinction is becoming increasingly irrelevant: living organisms look more and more like machines, and machines look more and more like living organisms
• The **natural/artificial distinction** is highly discussed in religion, genetic engineering, food production, virtual realities, computer intelligence, medicine etc.
=> here "natural" is mostly considered beneficial, safe, reliable and trustworthy while "artificial“ is basically considered imperfect, immoral, unhealthy, damaging and dangerous

Raymond Kurzweil's vision

• **Inventor and futurist**: optical character recognition (OCR), text-to-speech and speech recognition technology and electronic keyboard instruments
• **Author** of several books on artificial intelligence (AI), transhumanism, **the technological singularity**, and futurism
• Receiving many awards in including 15 (!) honorary doctoral degrees
• He made many **future (technology) predictions** while many of them became surprisingly reality

The technological singularity (predicted 2005):

2010-2020

• $1000 computers will have the **same processing power as human brains**
• Computers become smaller and increasingly integrated into everyday life (clothes, furniture...)
• **Glasses** that beam images onto the users' retinas to **produce virtual reality (VR)**
• VR glasses will also have built-in computers featuring "virtual assistant" programs that can help the user with various daily tasks (**augmented reality**)

2020-2030

• Computers less than 100 nm big will be possible
• **Nanomachines** are **used for medical purposes** (e.g., performing detailed brainscans)
• Nanobots capable of entering the bloodstream to "feed" cells and extract waste (no eating)
• Nanotech-based manufacturing will be in widespread use
• **Virtual reality** will be so high-quality that it will be **indistinguishable from real reality**
• A computer is a "Strong AI" and can think like a human

Kurzweil's prediction of a technological singularity

2030-2040

• **Mind uploading** becomes possible: Transferring and copying a complete human's mind
• Nanomachines inserted into the brain control incoming and outgoing signals
• As a result, **truly full-immersion virtual reality** could be generated without the need for any external equipment. Afferent nerve pathways could be blocked, totally **canceling out the "real" world** and leaving the user with only the desired virtual experience
• Brain nanobots allow humans to greatly expand their cognitive, emotional, memory and sensory capabilities, to directly interface with computers, and to "telepathically" communicate with other
• "Human body 2.0" consists of a nanotechnological system of nourishment and circulation, **obsolescing many internal organs**, and an improved skeleton.
• Human body 3.0: lacks a fixed, corporeal form and **can alter its shape and external appearance at will** via nanobot-based technology
• People spend most of their time in full-immersion virtual reality

2045-

The singularity

• Singularity occurs when artificial intelligences beat human beings as the smartest and most capable life forms on the Earth
• Technological development is taken over by the machines
• Machines enter into an **uncontrolled reaction of self-improvement cycles**, with each new generation of A.I.s appearing faster and faster
• From this point onwards, **technological advancement is explosive**, under the control of the machines, and thus cannot be accurately predicted

Kurzweil's prediction of a technological singularity

• The Singularity is an extremely disruptive, world-altering event that forever changes the course of human history
• The **extermination of humanity** by violent machines is **unlikely** (though not impossible) because sharp distinctions between man and machine will no longer exist (thanks to the existence of cybernetically enhanced humans and uploaded humans)
• A.I.s **convert more and more** of the Earth's **matter into engineered**, **computational substrate** capable of supporting more A.I.s. **until the whole Earth is one, gigantic computer**
• At this point, the only possible way to increase the intelligence of the machines any farther is to begin **converting all of the matter in the universe into similar massive computers**
• A.I.s radiate out into space in all directions from the Earth, breaking down whole planets, moons and meteoroids and reassembling them into giant computers
• This, in effect, **"wakes up" the universe** as all the inanimate "dumb" matter (rocks, dust, gases, etc.) is converted into structured matter capable of supporting life (though synthetic life)
• Machines might have the ability to make planet-sized computers by 2099, underscoring how enormously explosive technology will advance after the Singularity
• The process of "waking up" the universe could be complete as early as 2199
• With the entire universe made into a giant, highly efficient supercomputer, AI and human hybrids would have both supreme intelligence and physical control over the universe **including clearing the laws of Physics and interdimensional travel**

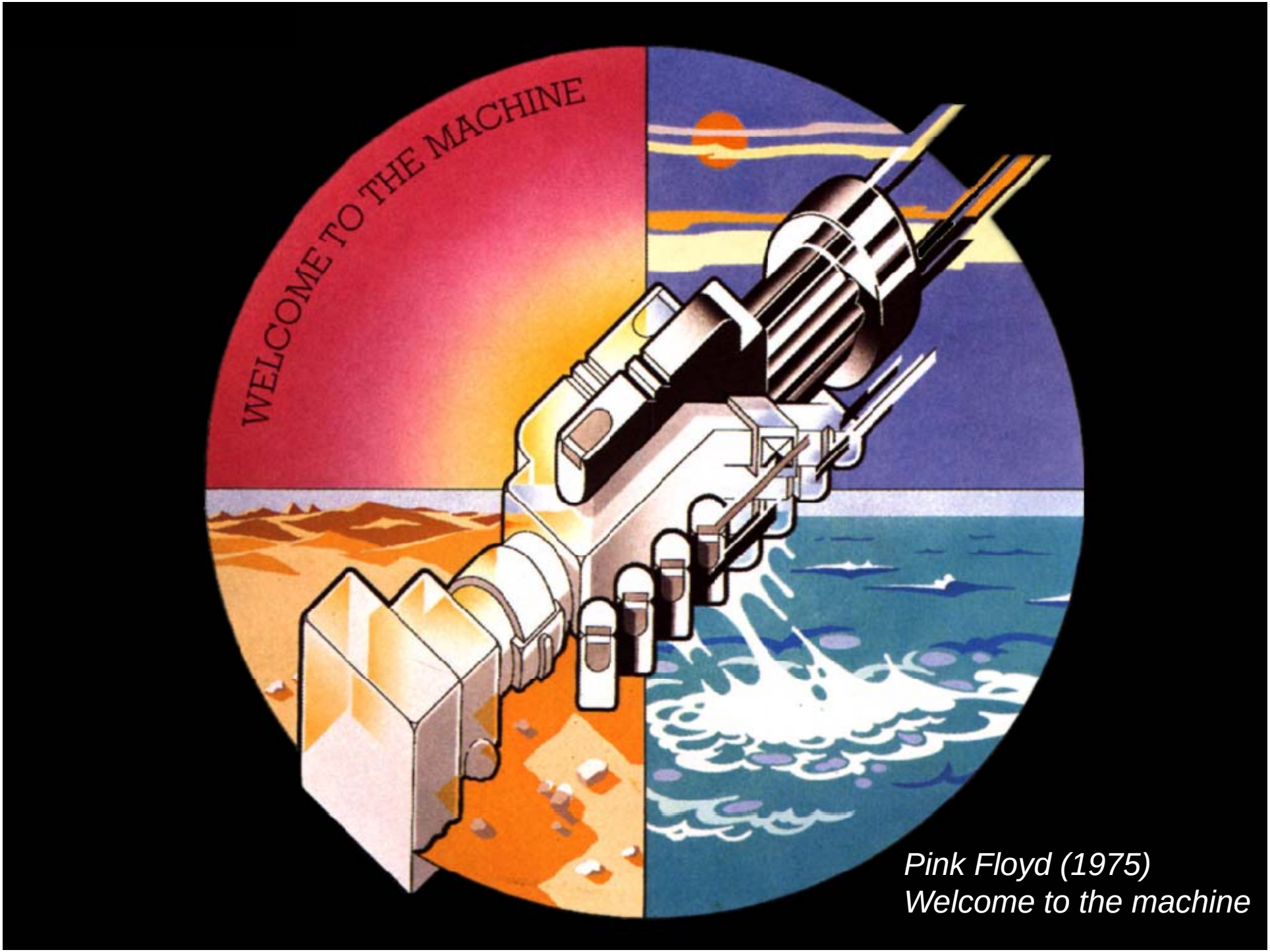
http://en.wikipedia.org/wiki/Ray_Kurzweil

The critiques

Douglas R. Hofstadter:
• "It's as if you took a lot of very good food and some dog excrement and mix it all up so that you can't possibly figure out what's good or bad".
• "It's an intimate mixture of rubbish and good ideas, and it's very hard to distinguish between the two, because these are smart people; they're not stupid."

Jaron Lanier (VR pioneer): "cybernetic totalism"

Bill Joy (cofounder of Sun Microsystems): Agrees with Kurzweil's timeline of future progress, but believes that technologies such as AI, nanotechnology and advanced biotechnology will create a dark, pessimistic, harmful and depressing (dystopian) world



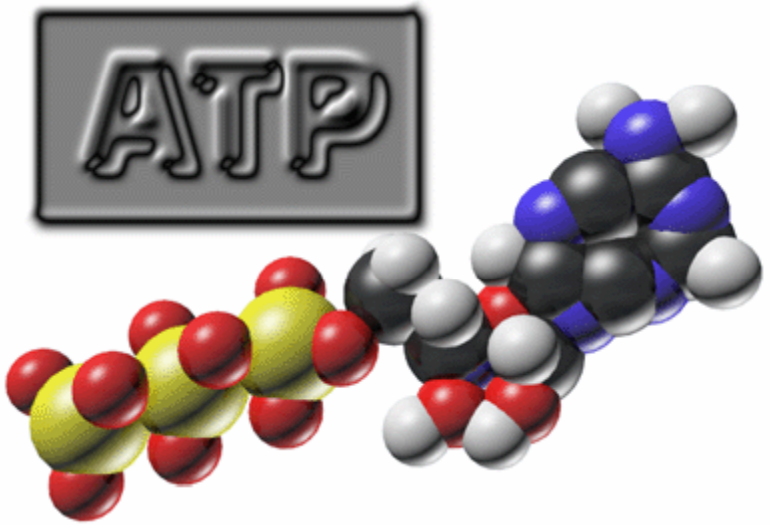
Pink Floyd (1975)
Welcome to the machine

ATP synthase: A molecular turbine

• Sunlight or nutrients (as glucose) are converted in the cell to a biologically universal energy carrier **ATP** (adenosine triphosphate) => the **fuel of the cell**

• During hydrolysis of ATP to ADP+Pi the cell can use the released energy to power many energetically unfavorable processes as:

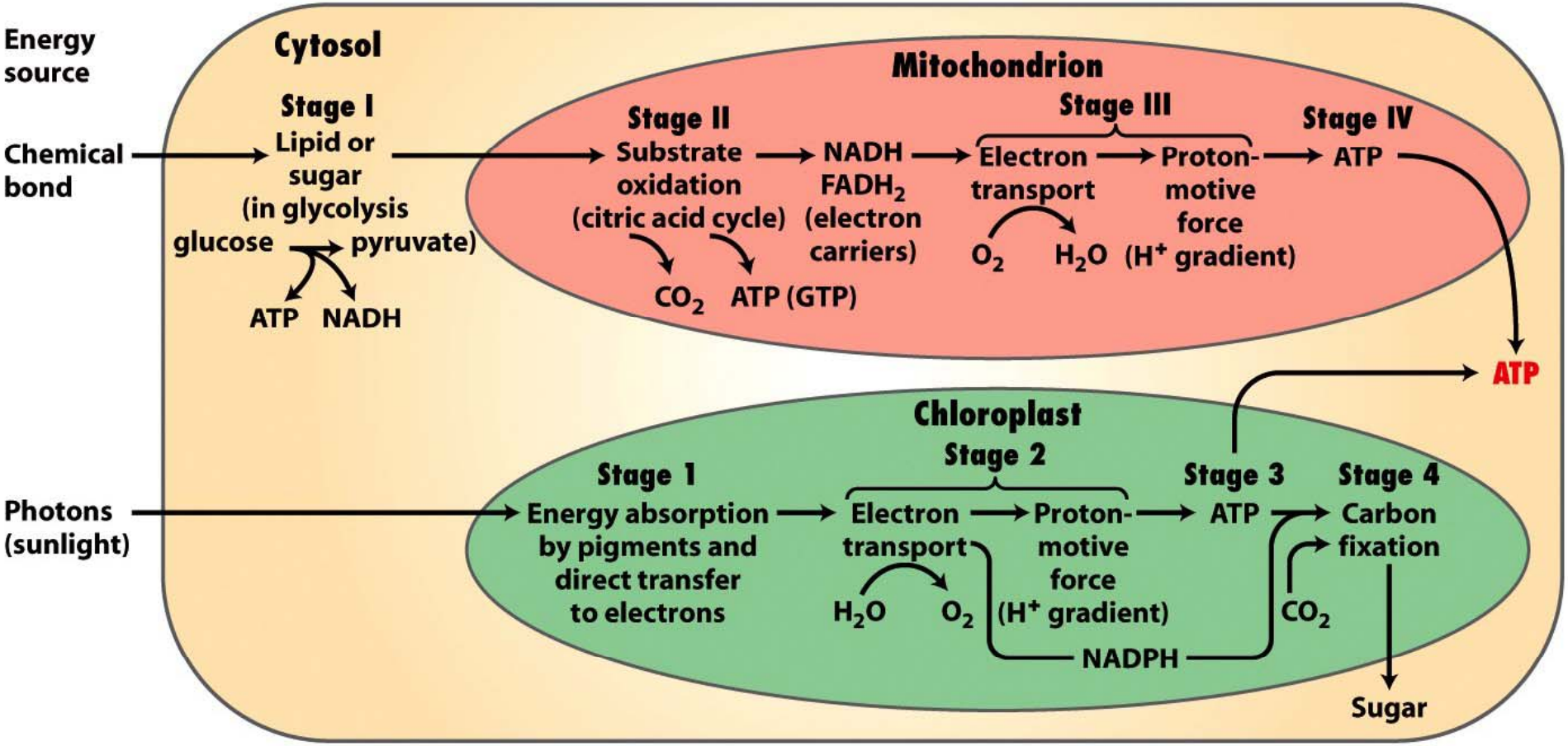
- **Protein synthesis** (from amino acids)
- **DNA synthesis** (from nucleotides)
- Molecule transport along a membrane via **ATP-powered pumps**
- Muscle contraction
- Cytoskeleton-based **molecular motors**
- Beating of **cilia and flagella** (moving of sperm and bacteria)



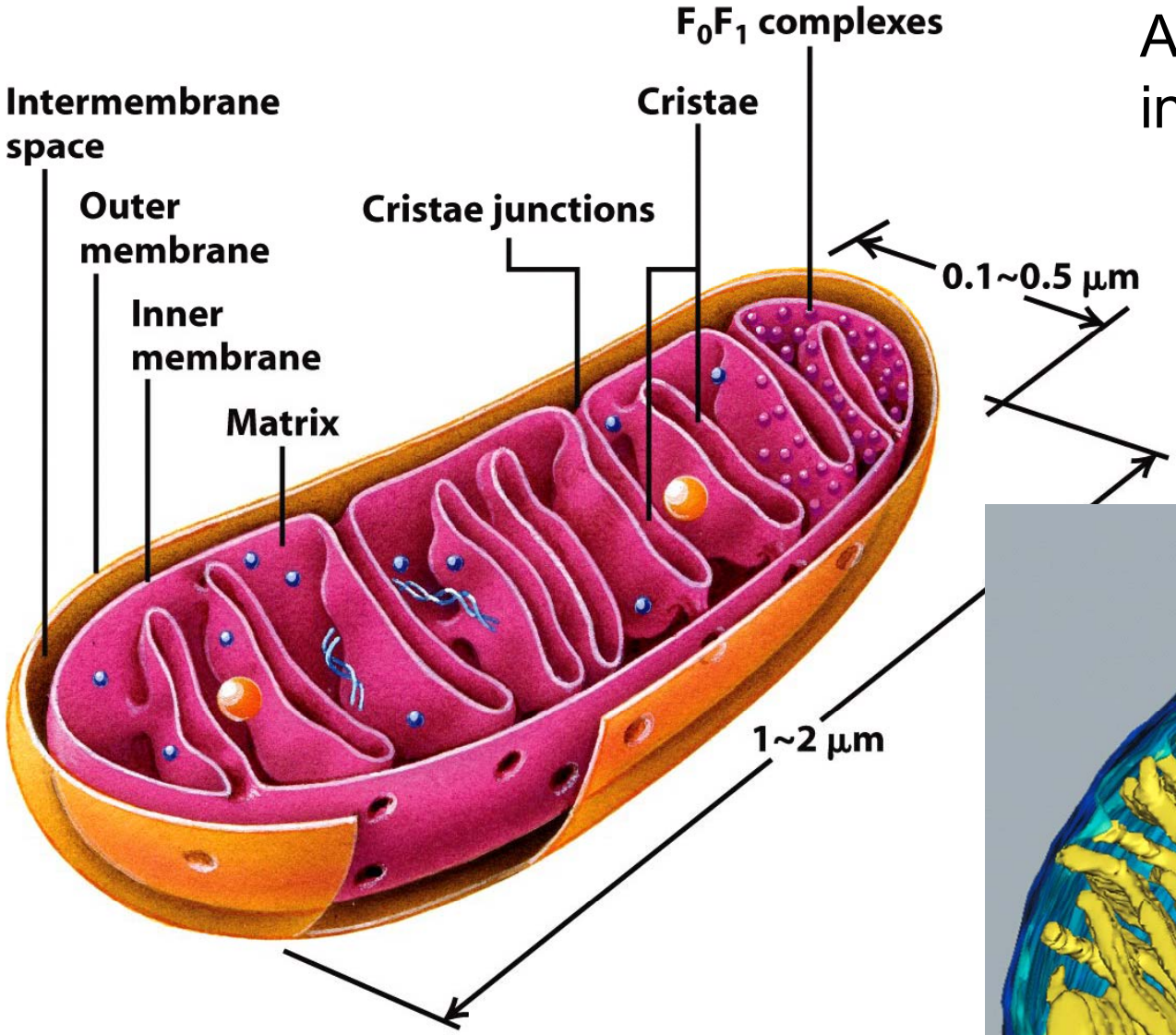
This guy was dreaming about?

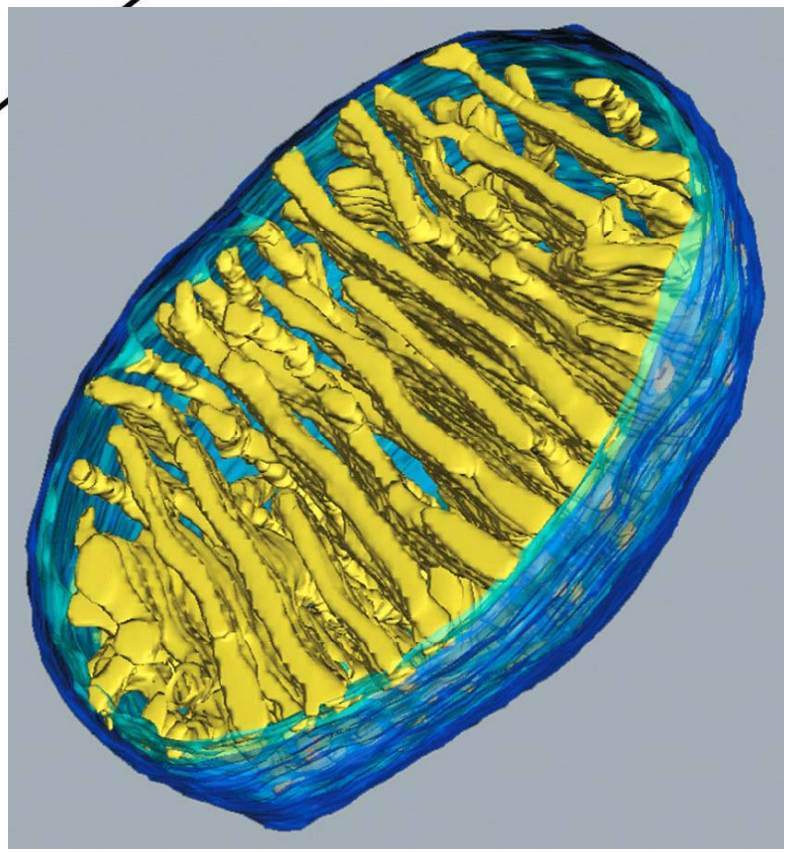
Molecular machines

In plants **ATP** is generated in chloroplasts using the photons from the sunlight
In animals **ATP** is generated in mitochondria by degrading sugars and lipids



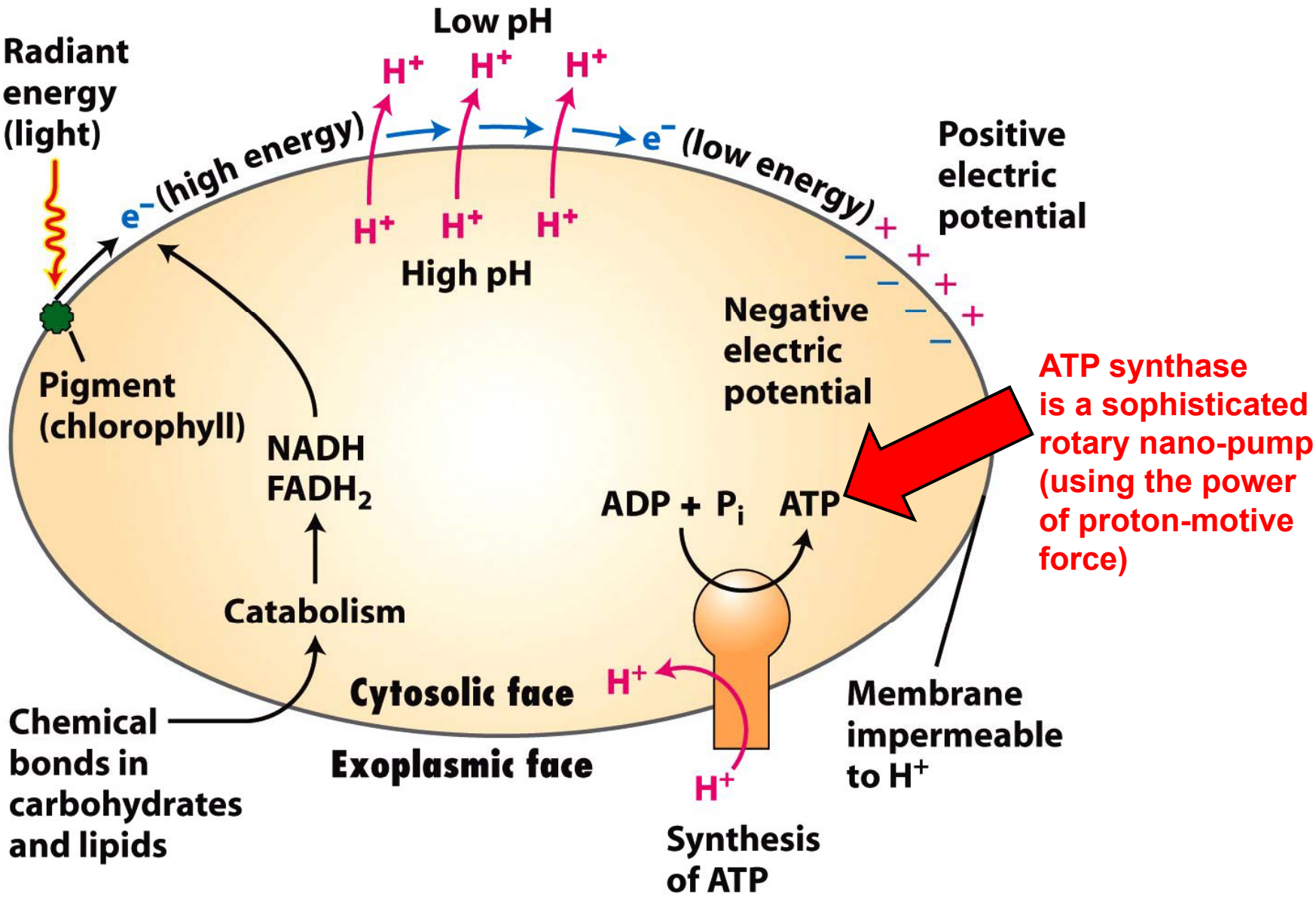
ATP is synthesized in the mitochondrion



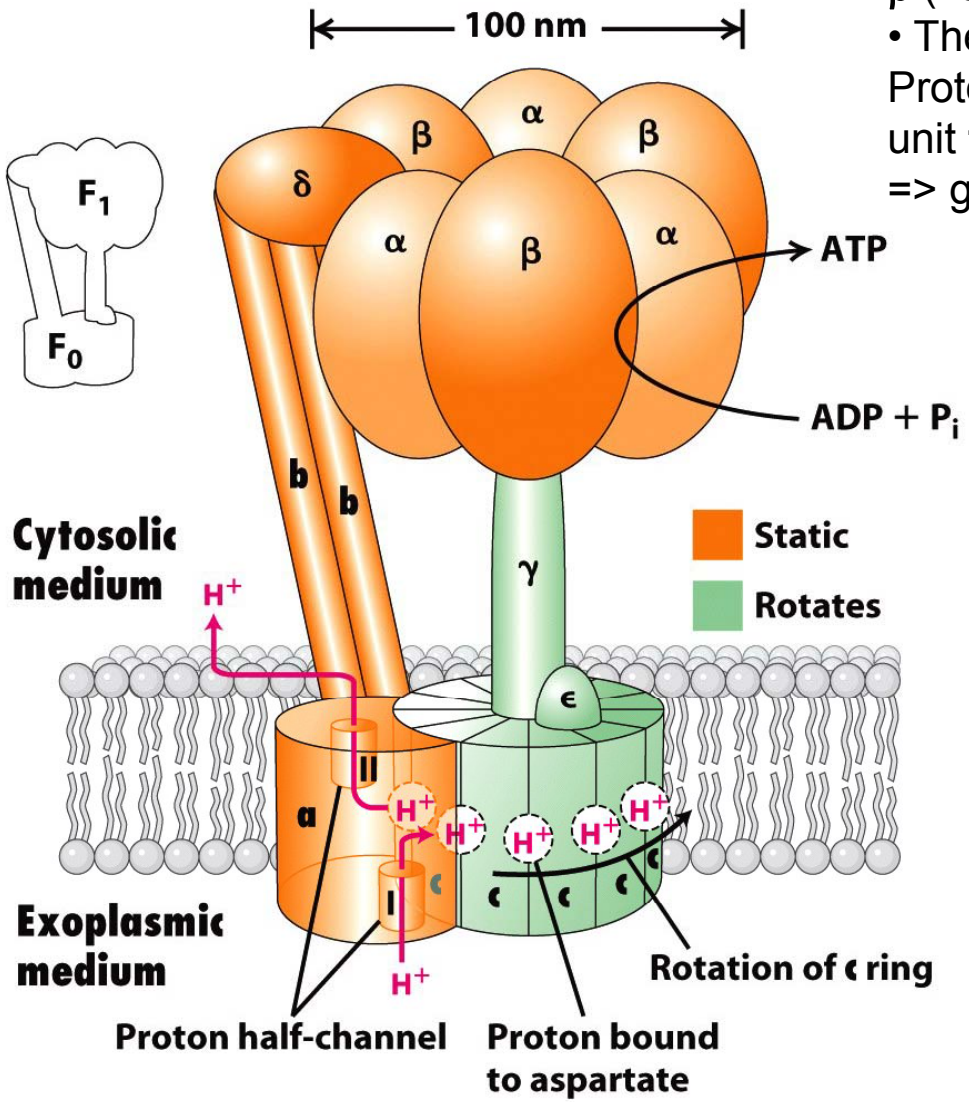


3D EM image of a mitochondrion (computer-generated from series of 2D EM images)

ATP is synthesized by a **rotary nano-pump** using the power of an proton gradient along the membrane (**proton-motive force**)



How does the ATP synthase (F_0F_1) work?



- ATPase consists of two major units: **F_0** and **F_1**
- **F_0** consists of subunits **a** (x1), **b** (x2) and **c** (x10)
- **F_1** consists of a **hexamer** composed of α (x3) and β (x3) subunits as well as of a γ, δ and ε subunits
- The **F_0** a-subunit contains two proton half-channels: Proton **channel I** guides a proton to a **c**-subunit => unit **turns** => proton of a preceding unit is released => guided thru half-**channel II** (released into cytosol)

- The δ **subunit** permanently links the hexamer to the F_0 unit
- Rotation of the c-subunit (and thus the connected γ subunits) causes a conformational change in the β subunits that **catalyzes ATP synthesis**
- The ATPase can make 400 ATPs per second! (134 rotations per second; one rotation needs 10 protons)

Animation
14_1_ATP_synthase.mov

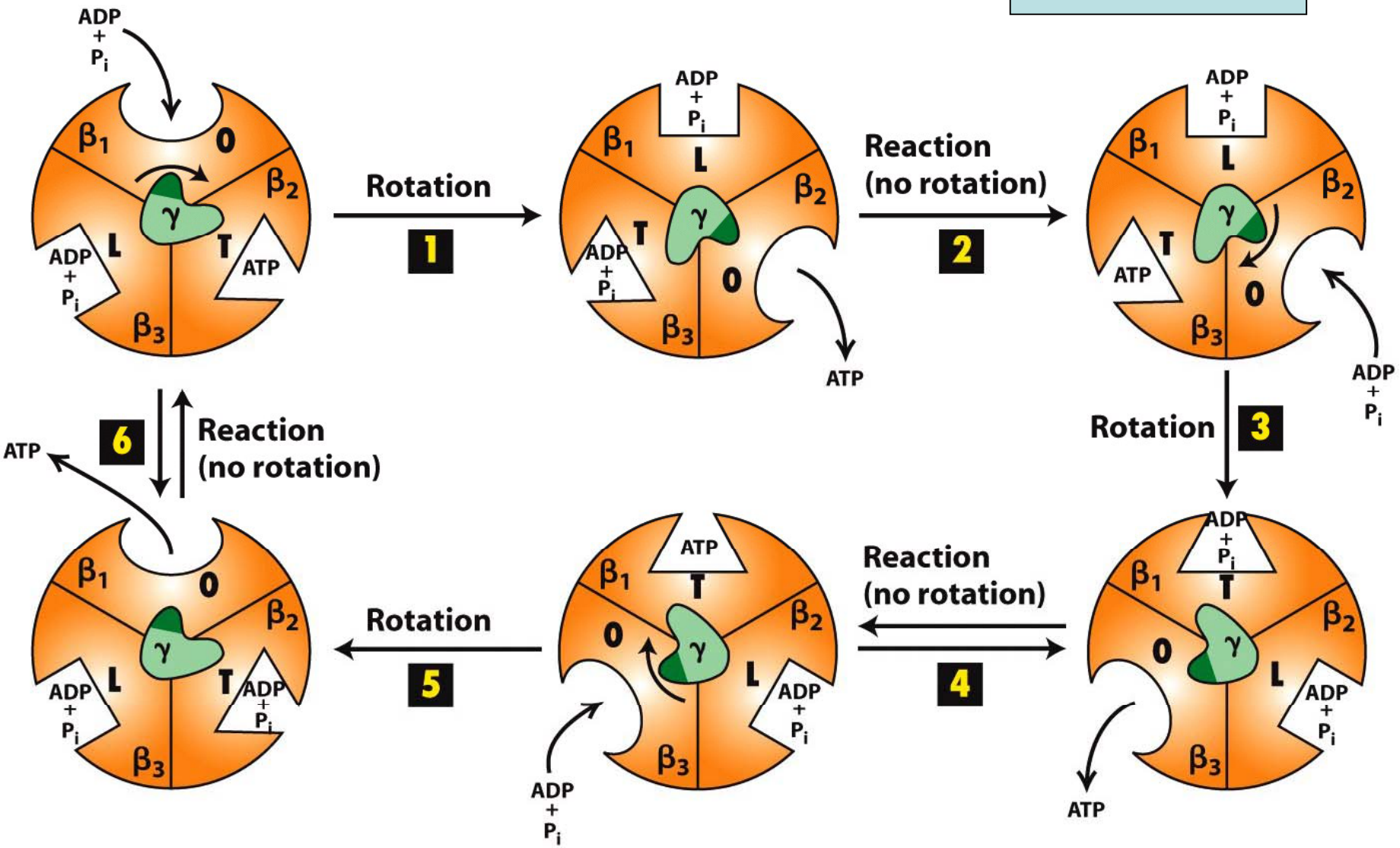
• Because the rotating F_0 γ subunit is **asymmetric**, it pushes differently to the F_1 β subunit which thus can appear in **3 different conformations**: O, L and T

• O (*open*) stage binds **weakly** ADP+Pi (or ATP)

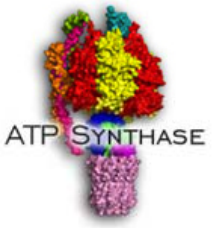
• L (*loose*) stage binds **strongly** ADP+Pi

• T (*tight*) stage favors the **chemical reaction** ADP+Pi => ATP

Animation
1203_ATP_synthesis.swf



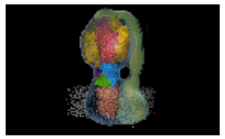
http://www.mrc-dunn.cam.ac.uk/research/atp_synthase/movies.php



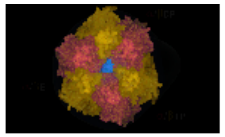
Animation
2_spheretop.mov

Movies

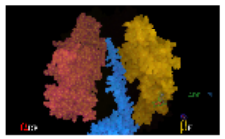
These movies were created by Said Sannuga in collaboration with John Walker and Andrew Leslie.



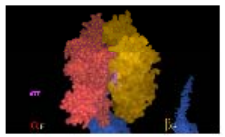
The rotary mechanism of mitochondrial ATP synthase. (12 Mb)



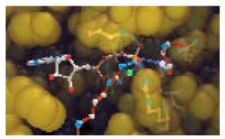
View from above and then below the F_1 domain along the rotating γ-subunit. (8.2 Mb)



How the rotating γ-subunit imposes the conformational states on a β-subunit required for substrate binding, ATP formation and ATP release. (4.5 Mb)



Three conformations of a catalytic β-subunit produced by 120º rotations of the central γ-subunit. (2.5 Mb)



Changes in the positions of sidechains in the catalytic site of F_1-ATPase bringing about binding and subsequent hydrolysis of ATP. (8.9 Mb)

> ATP Synthase Home
> Subunit Composition
> Rotary mechanism
> Structural analysis
> Current projects
> Group Leader - Sir John Walker
> Collaborators
> Current members
> Vacancies
> Recent publications

MRC | Dunn Human Nutrition Unit

14.2 ATP SYNTHASE—DISCO

Subunits:
- Center (gamma subunit): **Toyoki Amano**
- Left (beta subunit 1): **Hiroyuki Noji**
- Right (beta subunit 2): **Satoshi P. Tsunoda**
- Back (beta subunit 3): **Masaki Shibata**

Dance direction:
- **Nagatsuta Bon-Odori**

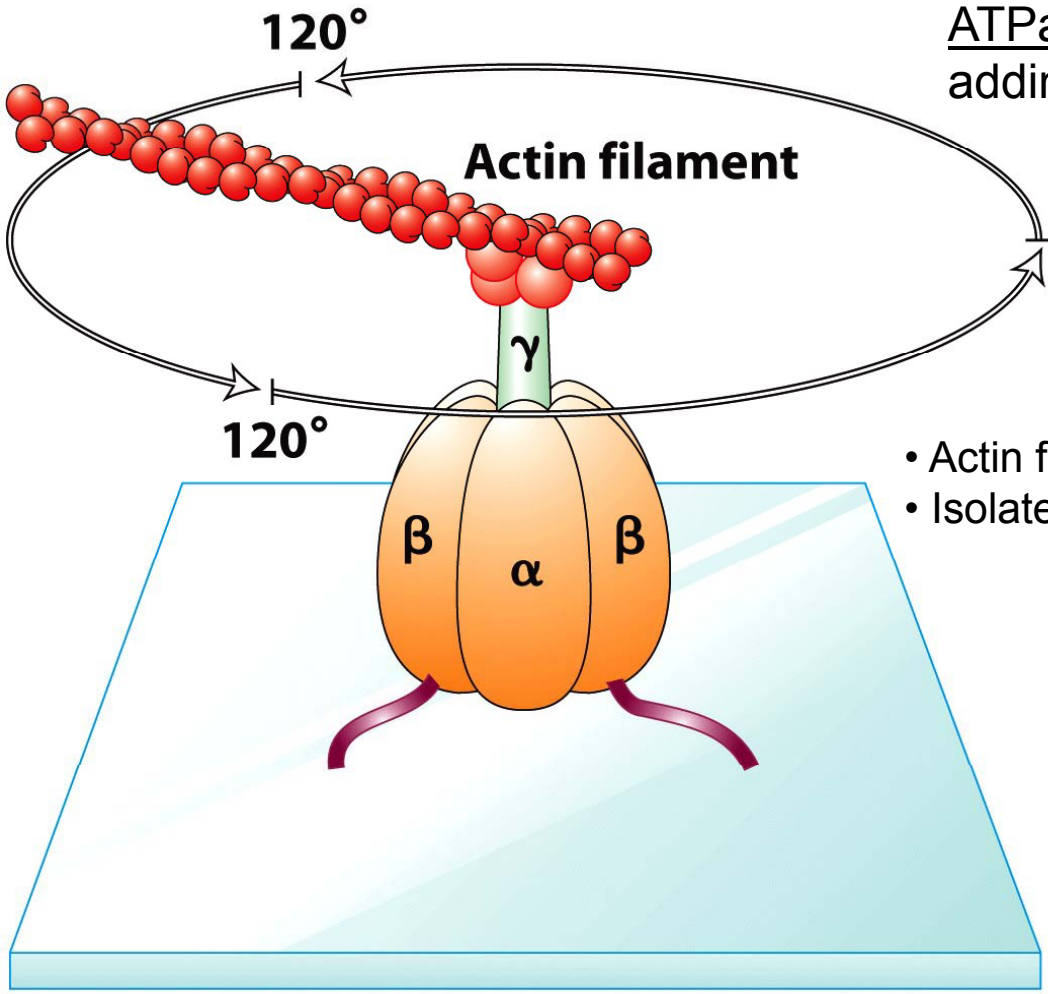
Camera work and production:
- **Hiroyuki Noji**

Animation

14_2_ATP_synthase_disco.mov

Noji et al., 1997, ***Nature***
Yasuda et al., 1998, ***Cell***

} Simple, but amazing experiment: **Making the rotation** of the ATPase **visible** (in nature and <u>real-time</u>) by sticking an actin polymer to the γ-subunit of the F_1 complex.

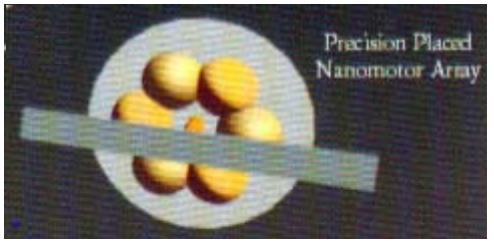


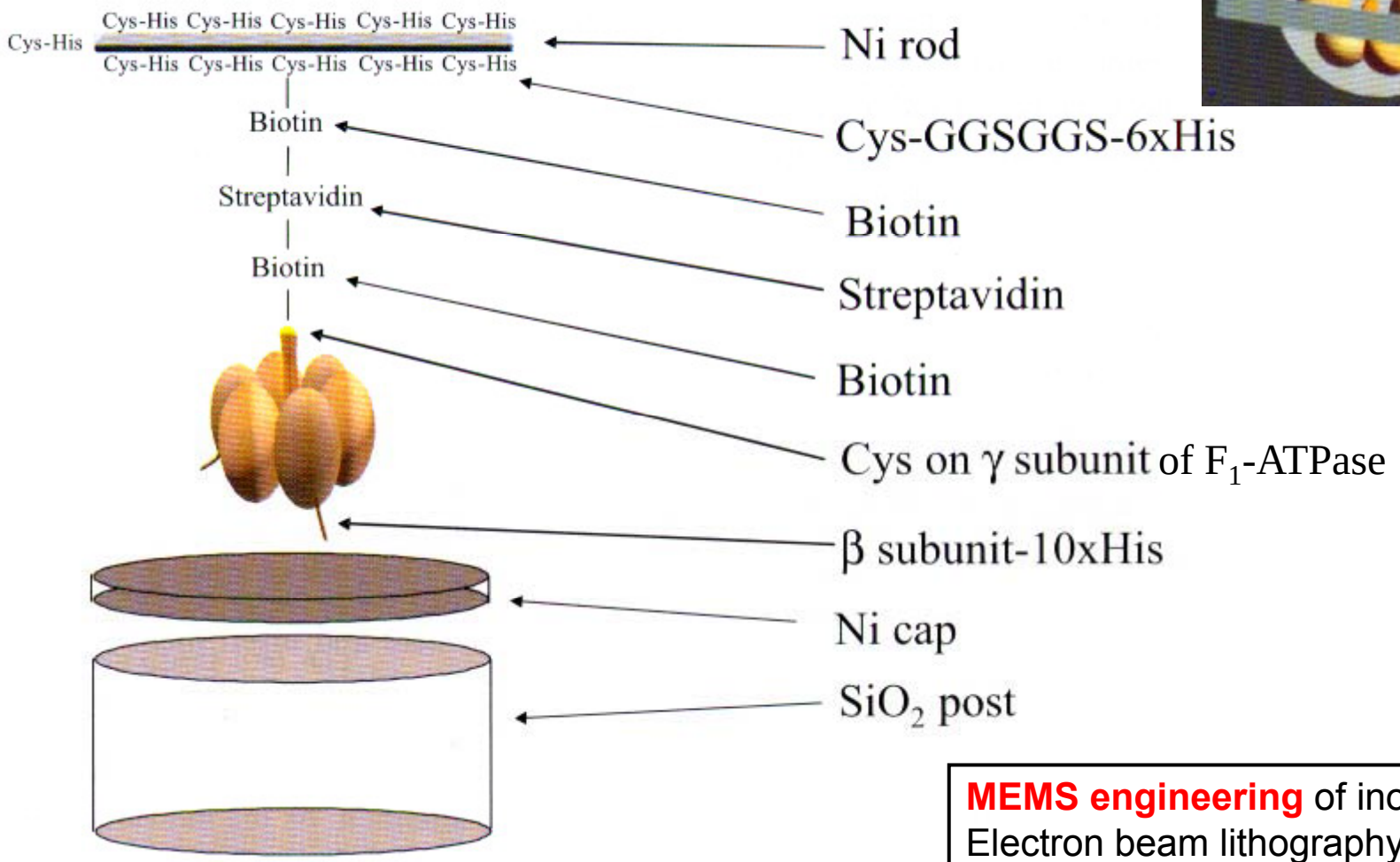
<u>ATPase works reversible</u>:
adding ATP makes it rotate

- Actin filament was fluorescently labeled
- Isolated F_1 complex adheres to a glass slide

Animation
1203_ATP_synthase_actin.mov

A hybrid nanodevice (nanopropeller)





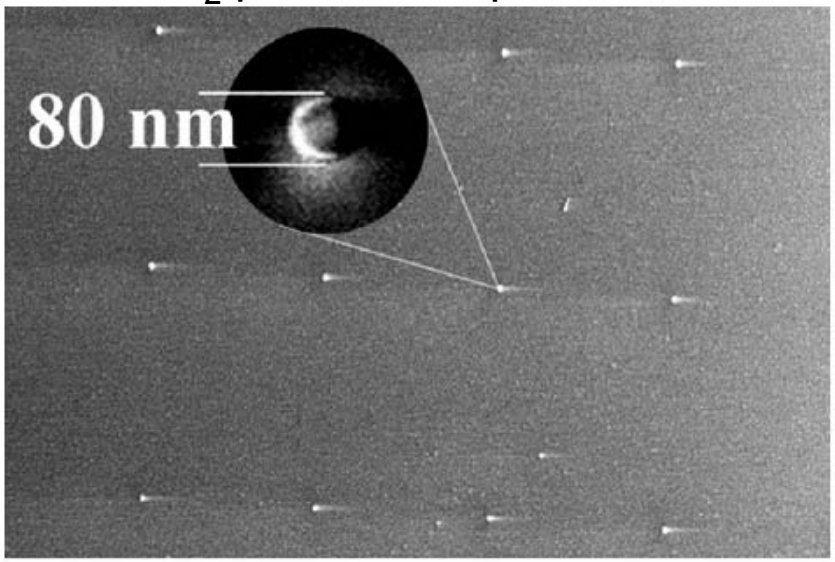
- **Biotin** (= Vitamin H) **binds strongly to** the protein **streptavidin** (strongest known ligand-protein interaction: K_D 10^{-15} = *almost covalent properties*)
- Negatively charged His binds to positively charged Ni
- **Biotin** strongly **interacts with cys-residues**

MEMS engineering of inorganic parts: Electron beam lithography, metal evaporation, reactive ion etching

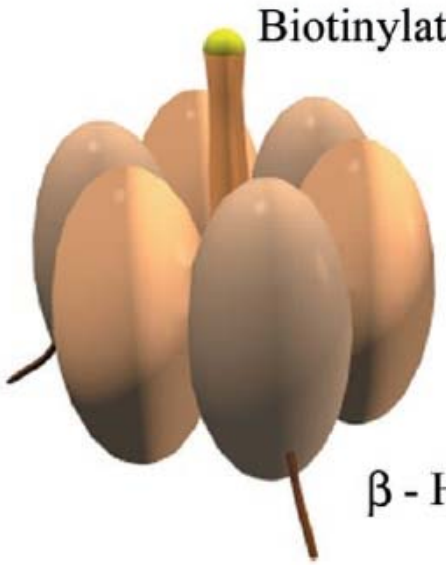
Protein engineering: Recombinant DNA technology to add 10x His on β subunit and Cys on γ subunit of F_1-ATPase

Nanofabrication of single parts for the motor

A SiO_2 post + Ni cap

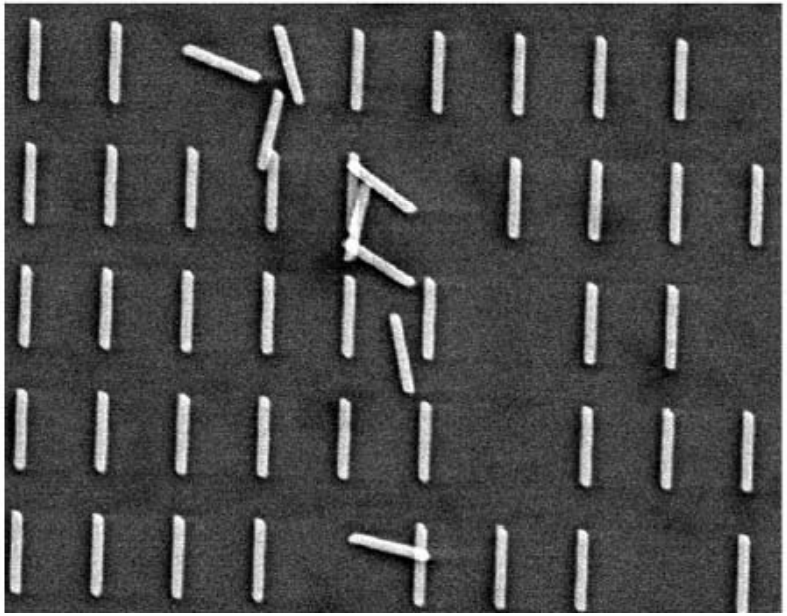


B

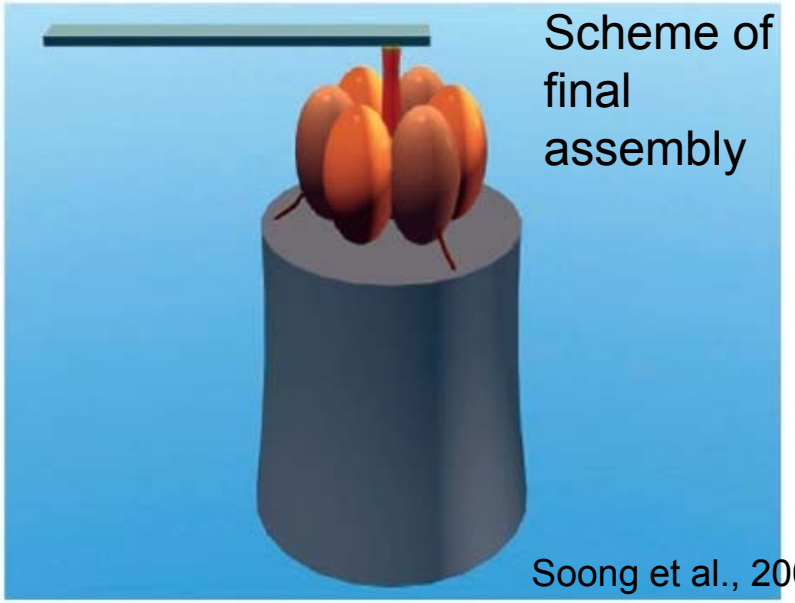


With tags (interfaces) modified motor

C Nanopropeller

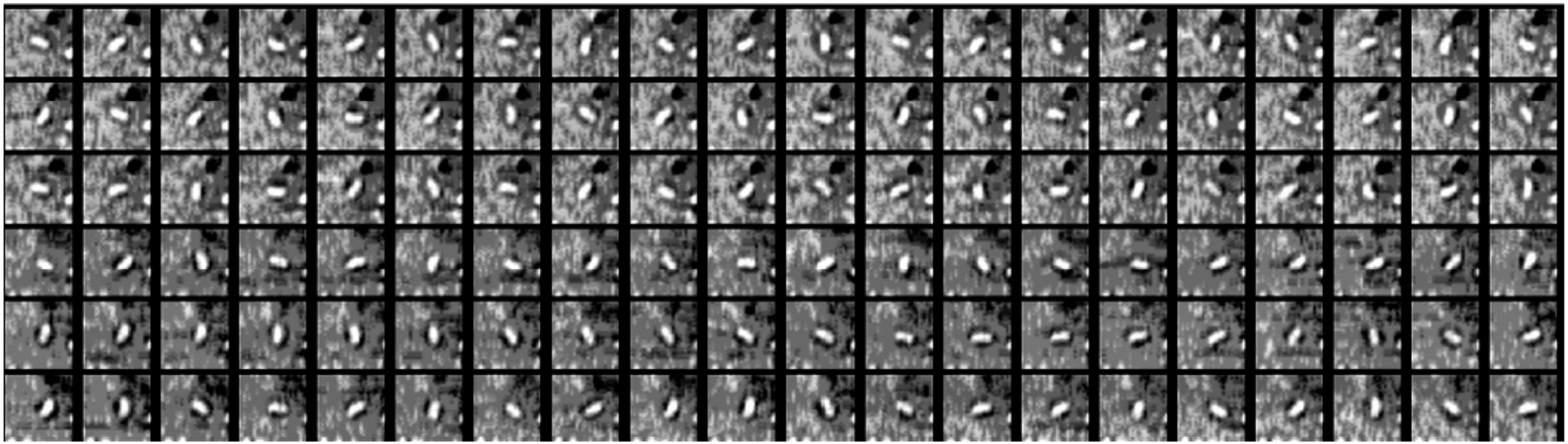


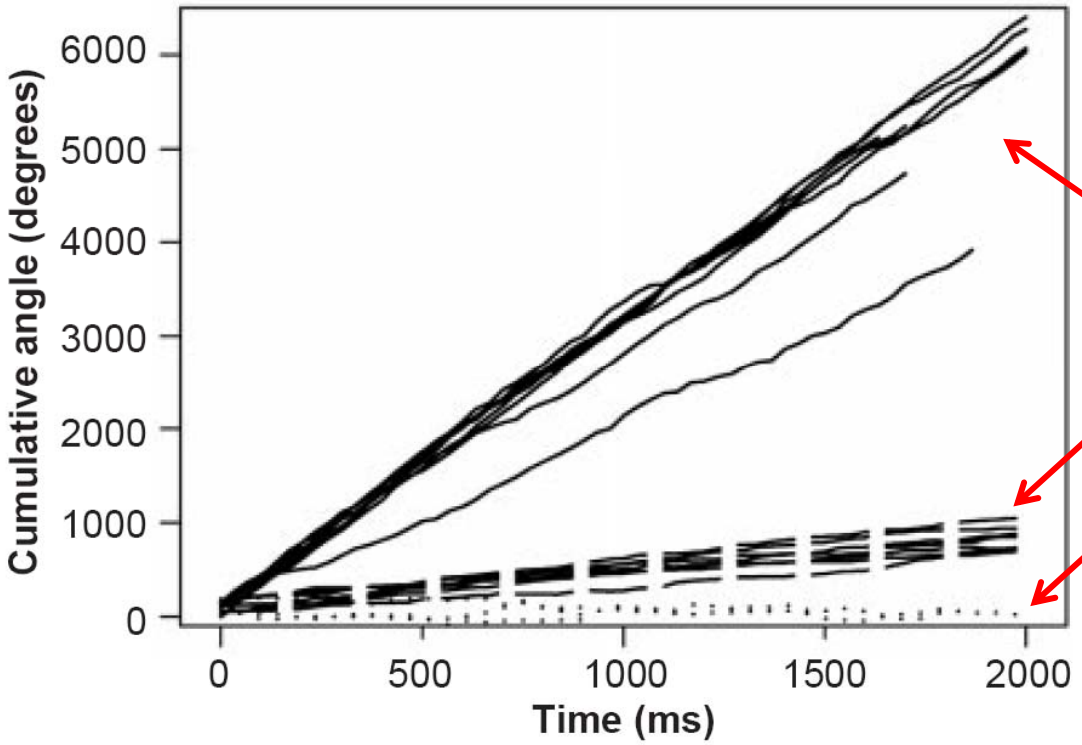
D



Soong et al., 2000, *Science*

Real-time recording of nanopropeller rotation





Propellers rotated for almost 2.5 hours

750 nm long propeller

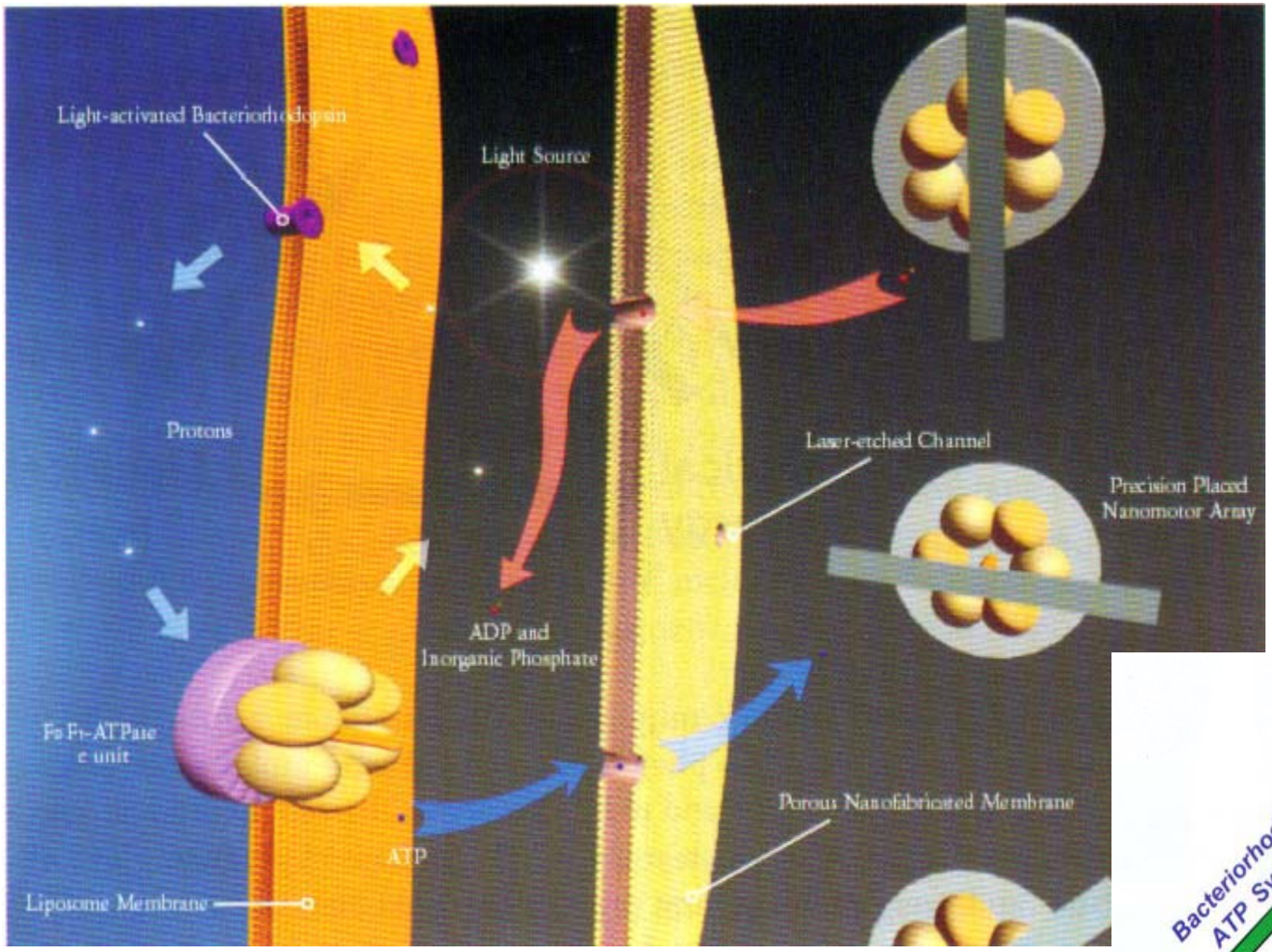
1400 nm long propeller

1400 nm long propeller + sodium azide (NaN_3)

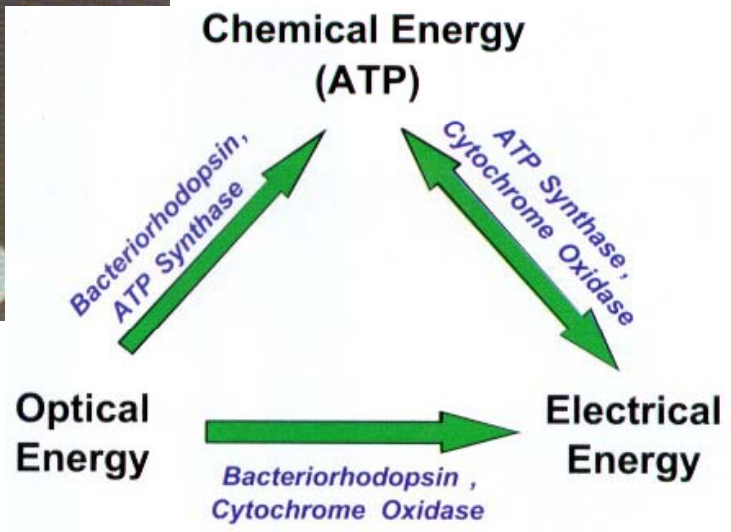
Soong et al., 2000, *Science*

A self-fueled hybrid nanodevice

ATP-regenerating system using **bacteriorhodopsin** (BR), **light** and a **ATP-synthase**



- **BR** pumps H^+ after absorption of *photons*
- **ATPase** uses proton-gradient to produce ATP from $ADP{\cdot}P_i$
- ATP powers **hybrid nanodevice**
- $ADP{\cdot}P_i$ diffuses thru porous nanofabricated membrane back to ATPase

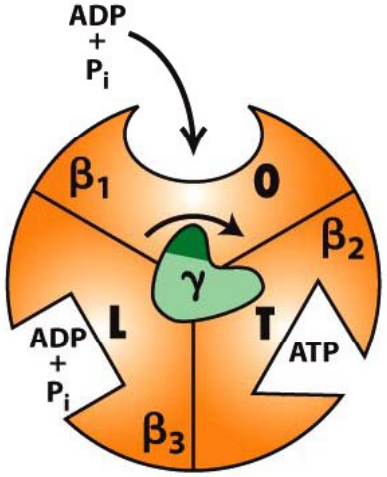


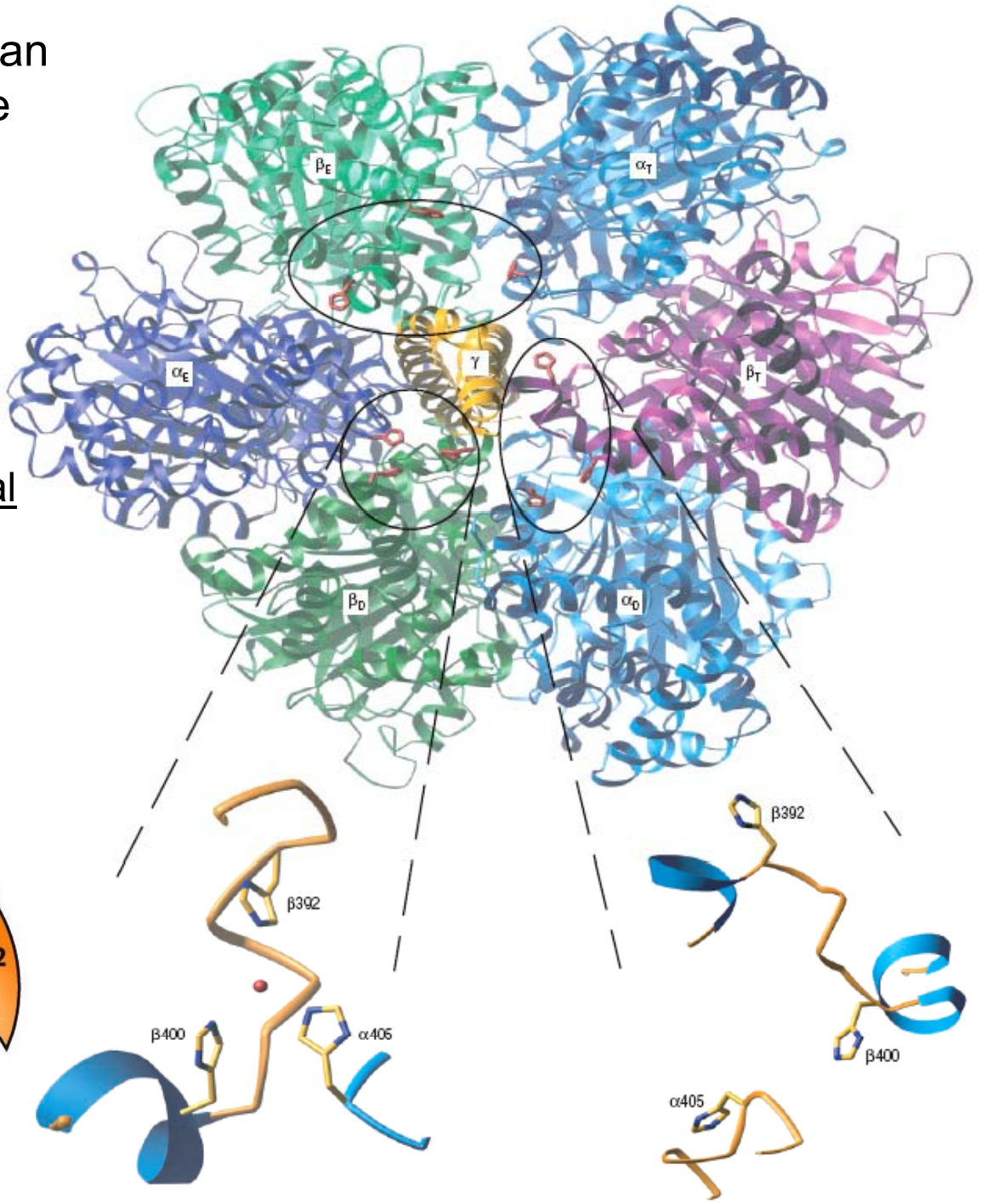
Schliwa, Molecular Motors, 1st Ed.

Protein engineered design of an on/off switch in the F_1-ATPase

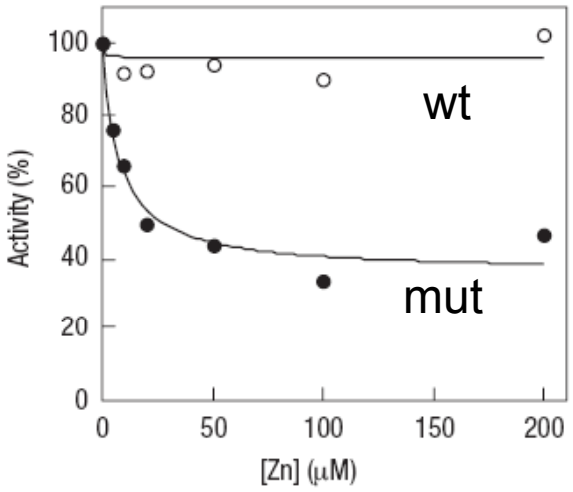
How to turn the motor on and off in the constant presence of ATP supply?

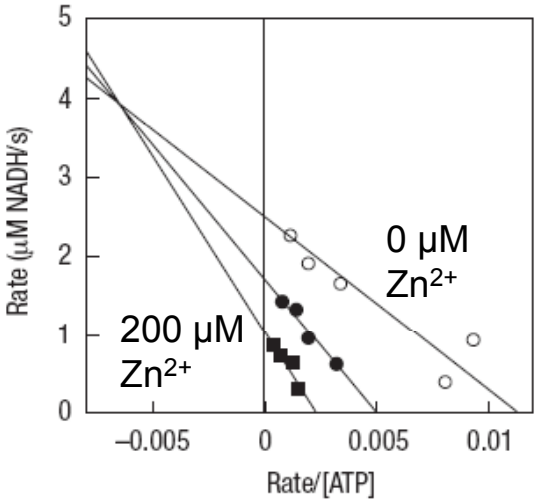
- Engineering "artificial" allosteric inhibition sites on the β subunits:
  ⇒ adding **His-tags** for binding of
  ⇒ **Zn^{2+}** to suppress conformational changes during γ subunit rotation
- Reversing the effect: adding a Zinc chelator (**phenanthroline**)

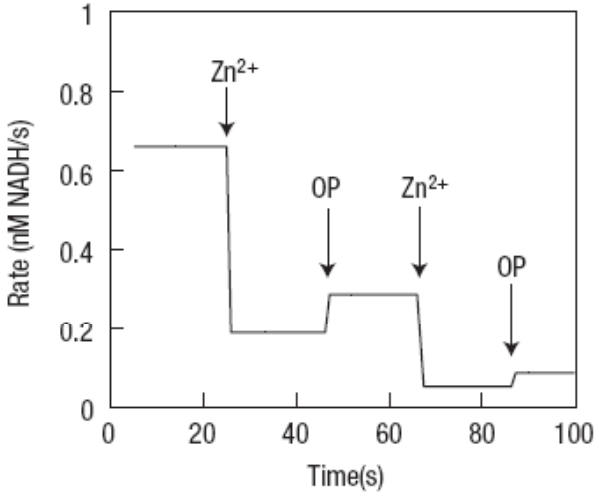


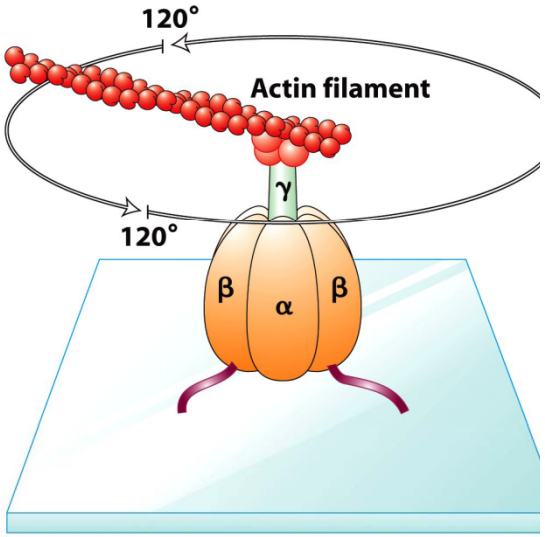


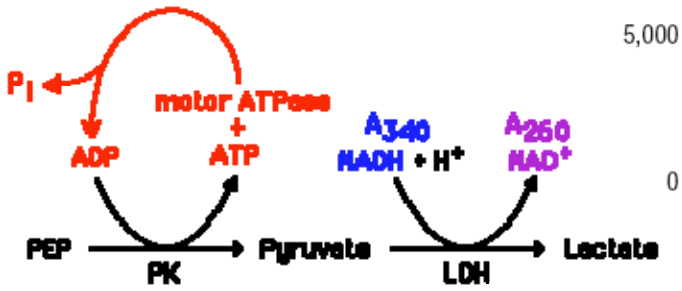
Allosteric inhibition by Zn^{2+} stops both, motor and enzyme activity

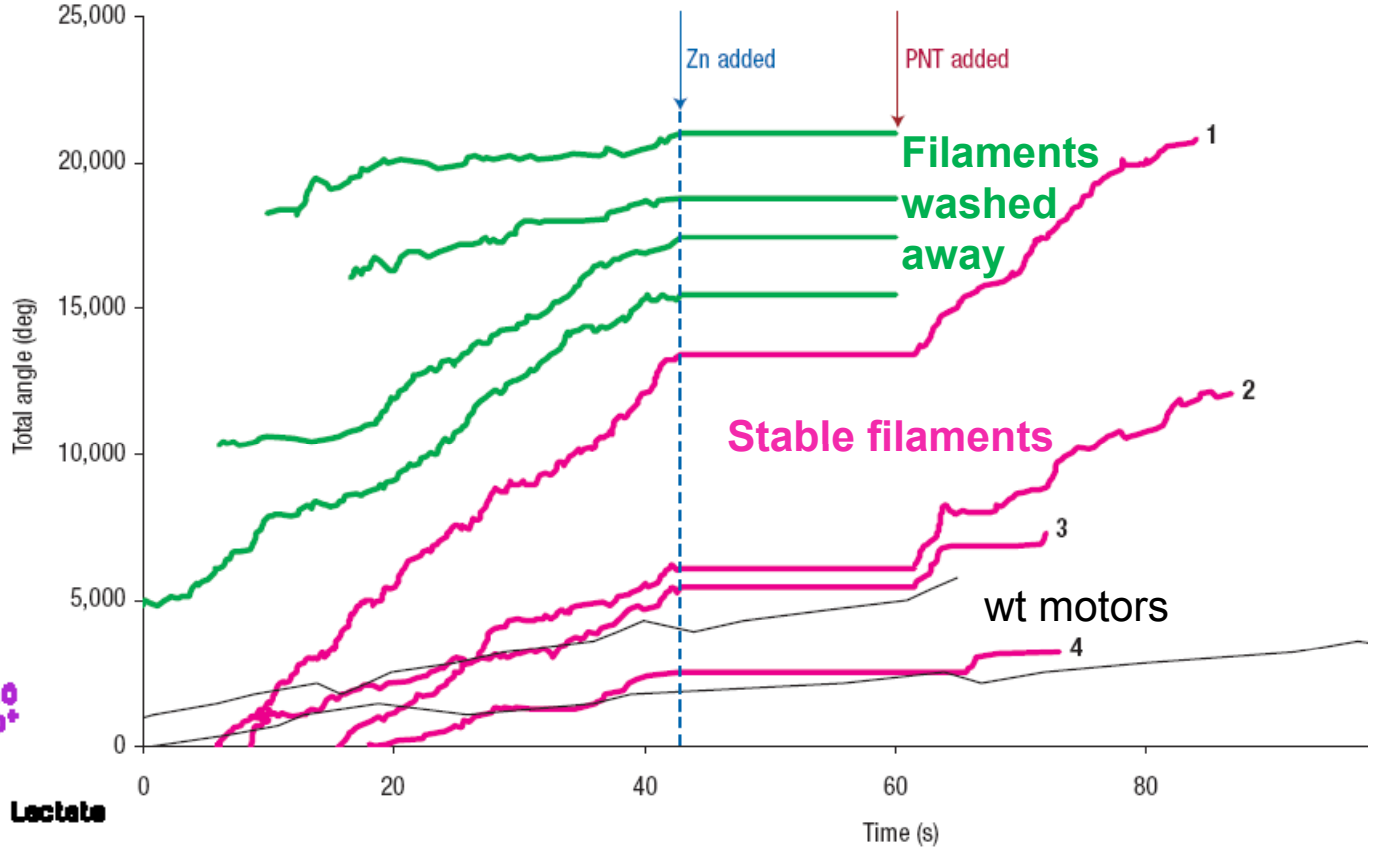












A nano-biomachine powered by highly motile bacteria

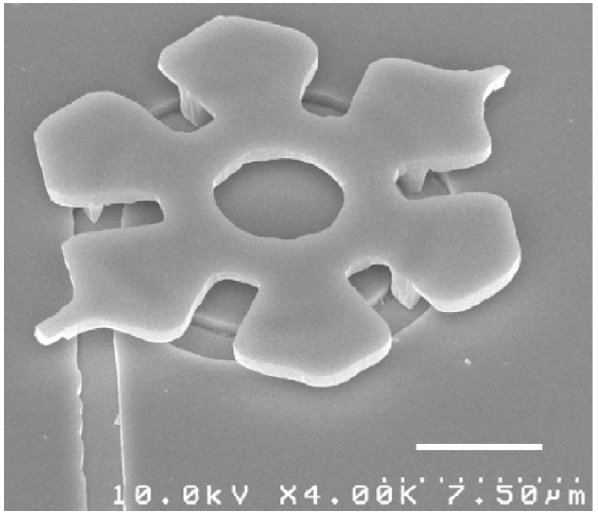
• Highly motile **gliding bacteria** *Mycoplasma mobile* pulled on a microrotor fueled by glucose

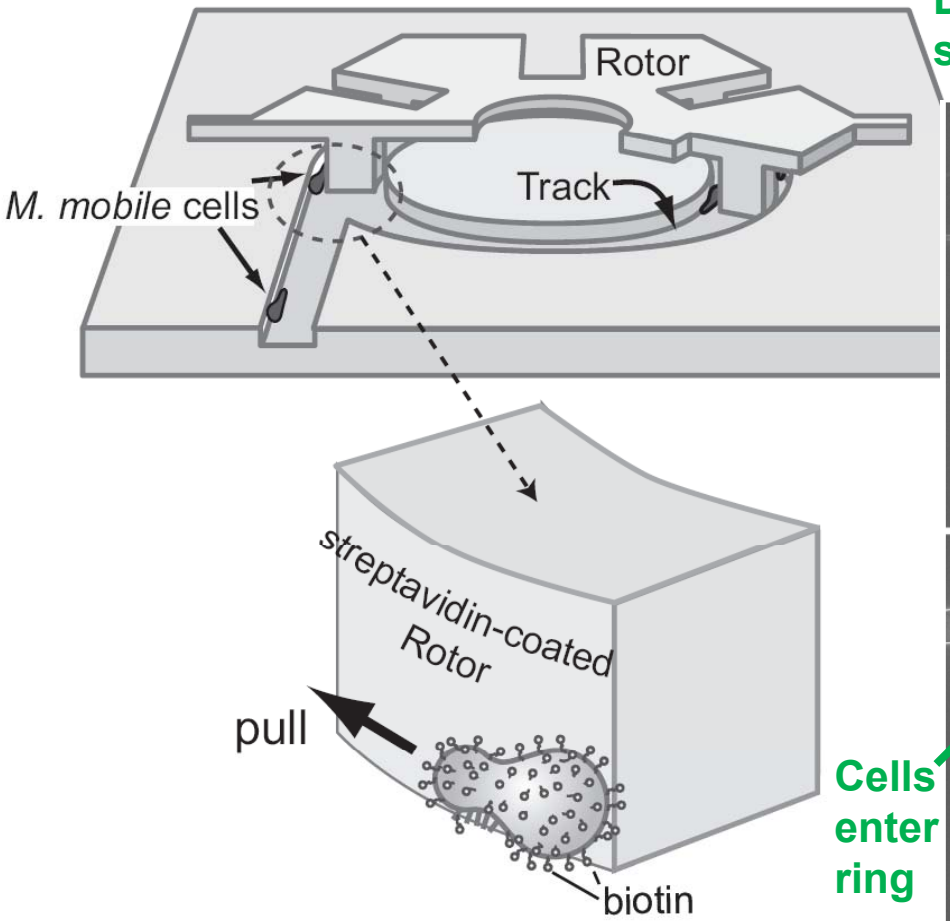
• Achieving of **unidirectional movement**:

➢ Asymmetric floating of cells into the circular track

➢ Glycoprotein coating on track-bottom required for cell attachment

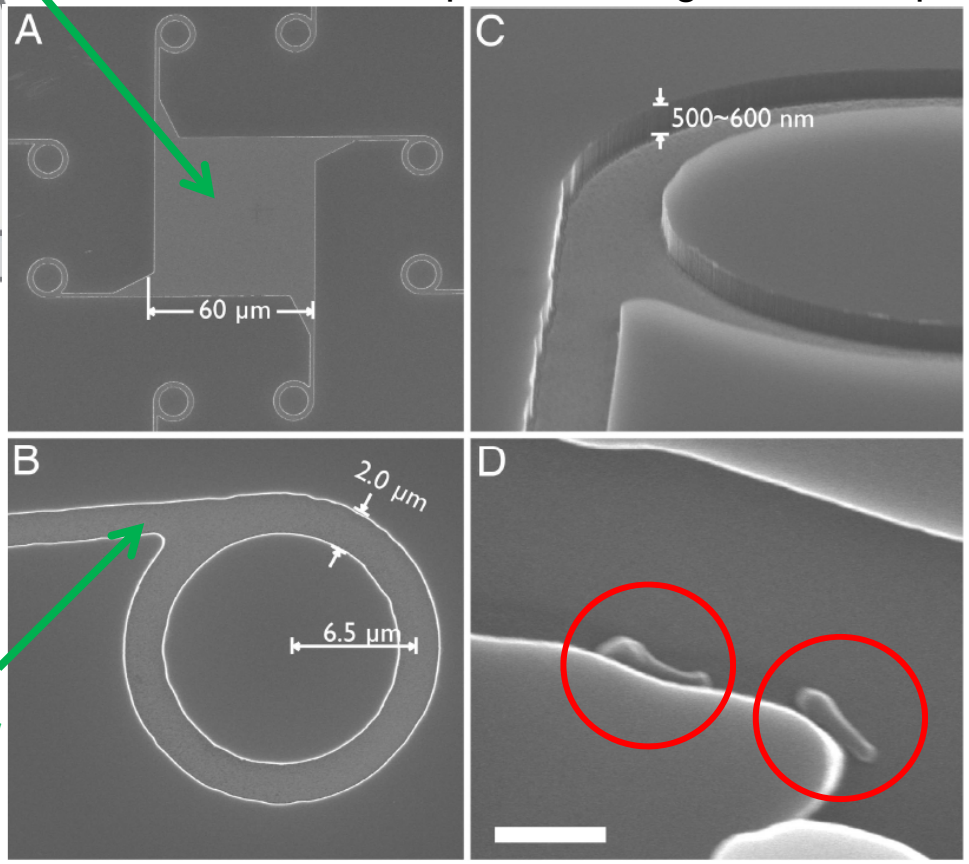
➢ Restricting **biotin-labeled bacteria** movements to streptavidin-coated rotor





Drop bacteria solution here

Rotor placed in ring in a 2nd step



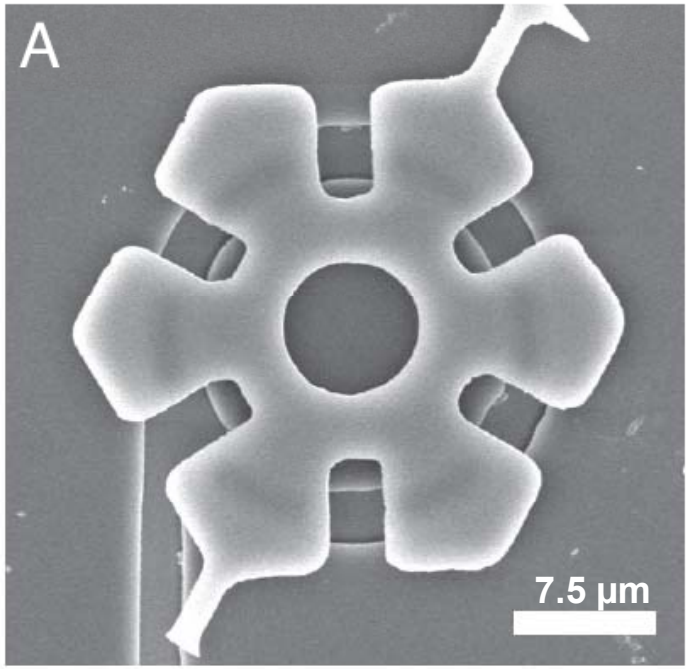
Cells enter ring

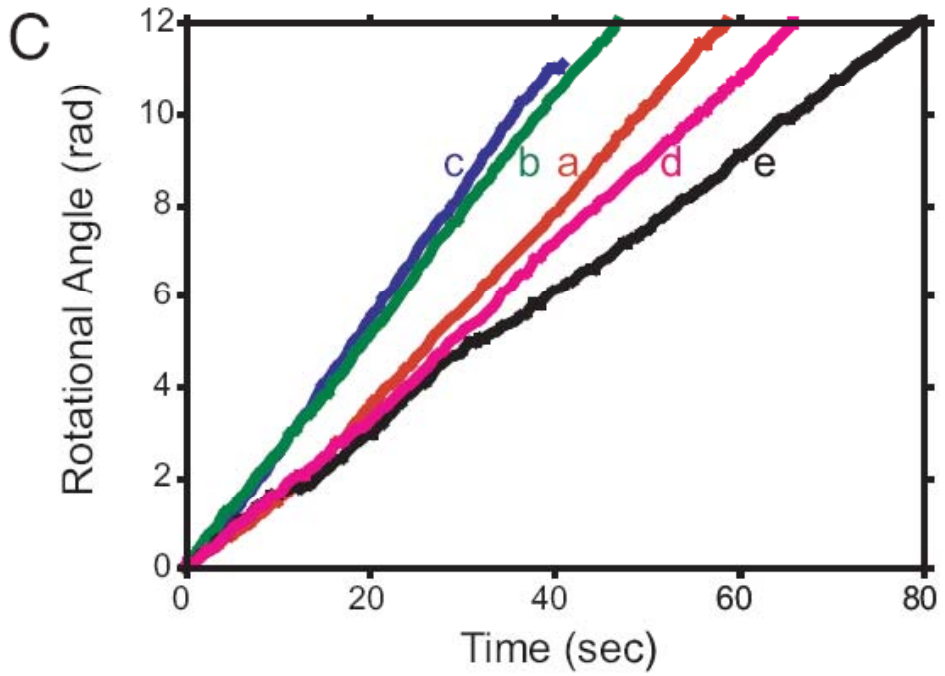
A nano-biomachine powered by highly motile bacteria

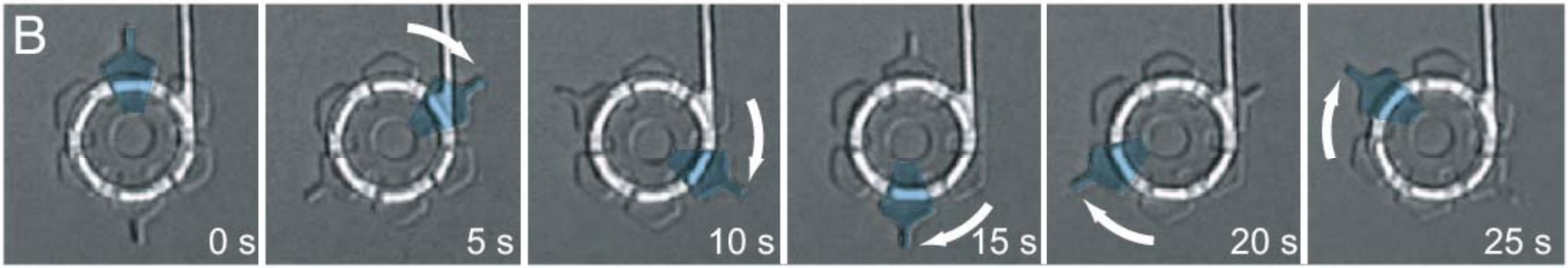
Speed: 3 rpm
Torque: 2-5 x 10^{-16} N·m
Stall force: 27 pN

Movie
bacteria powered microrotor.mov

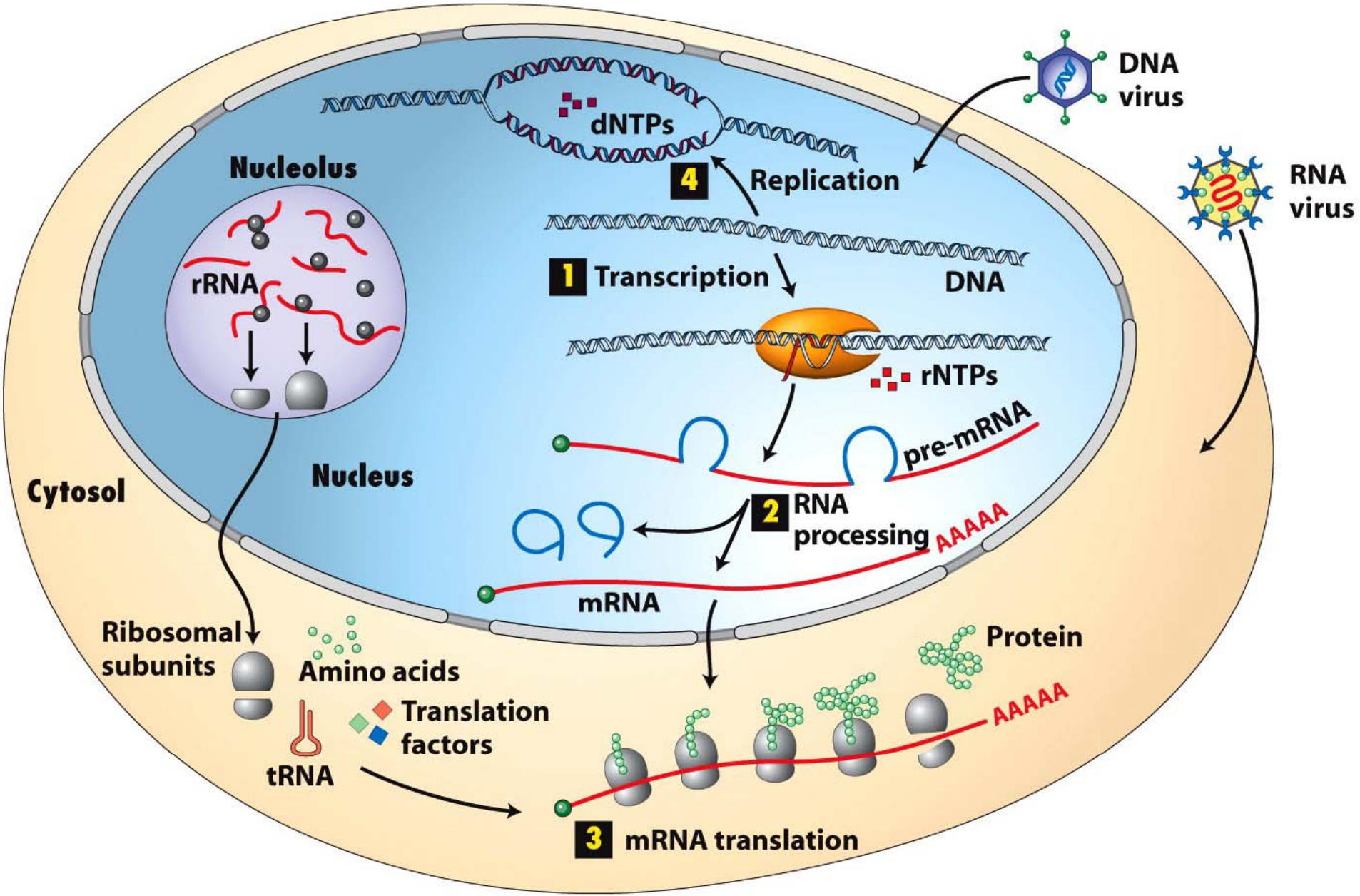
Movie
bacteria powered microrotor_B.mov



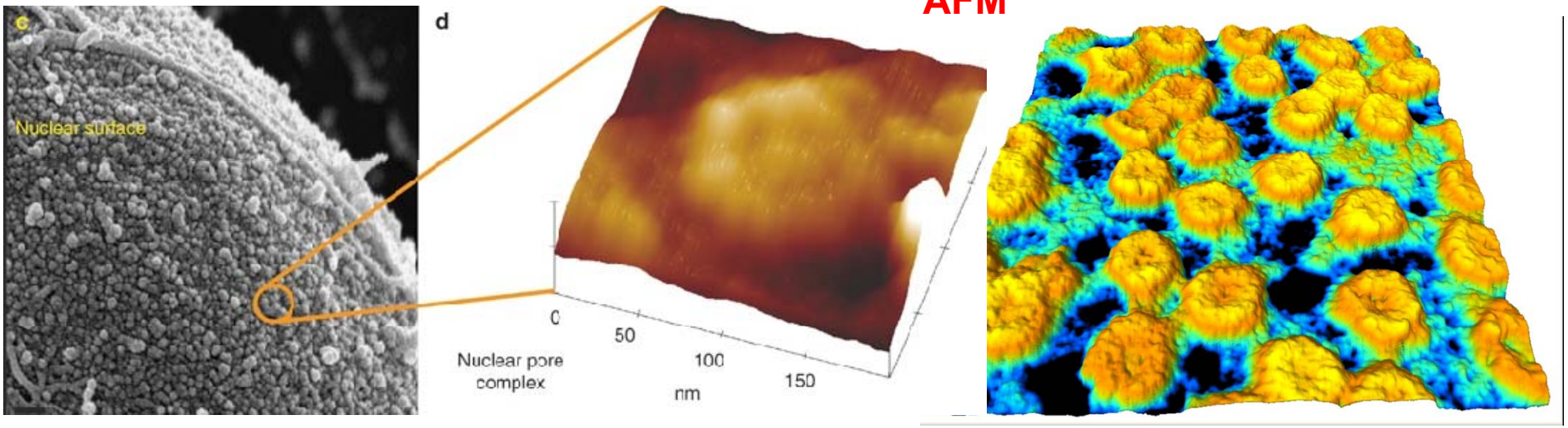


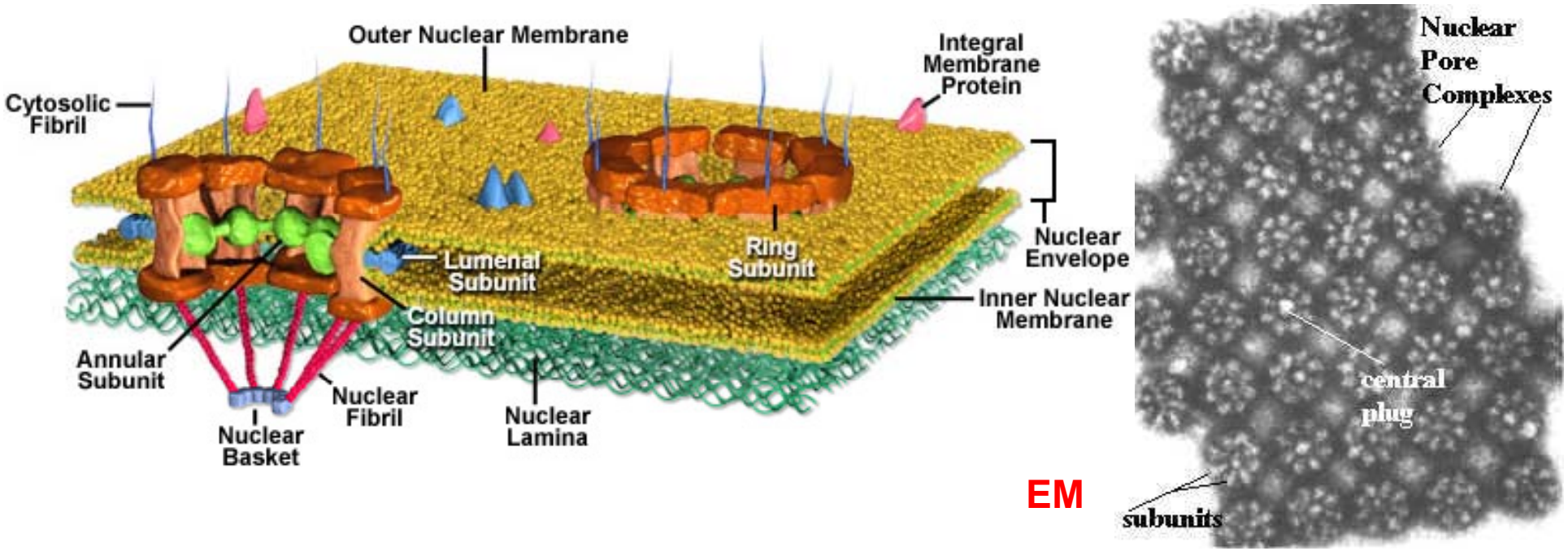


The nuclear pore: a molecular filter

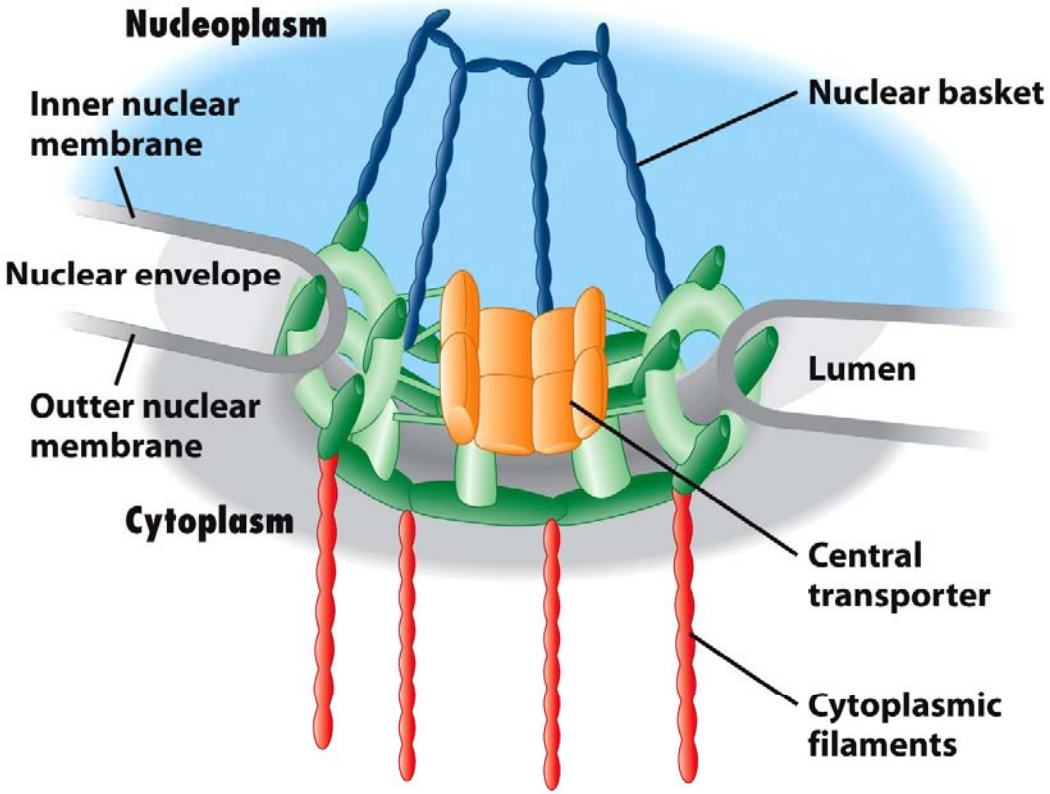


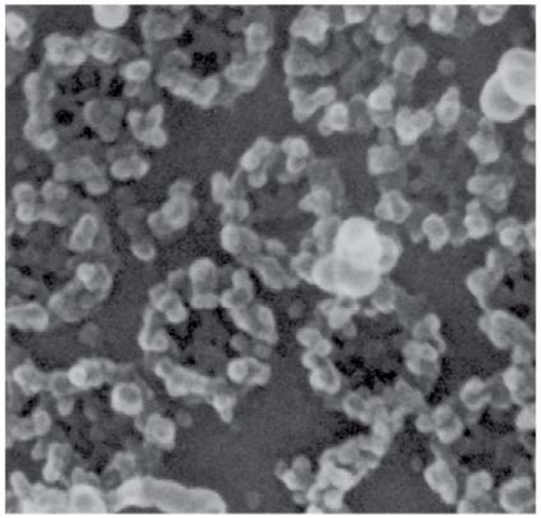
The nuclear pore: a molecular filter

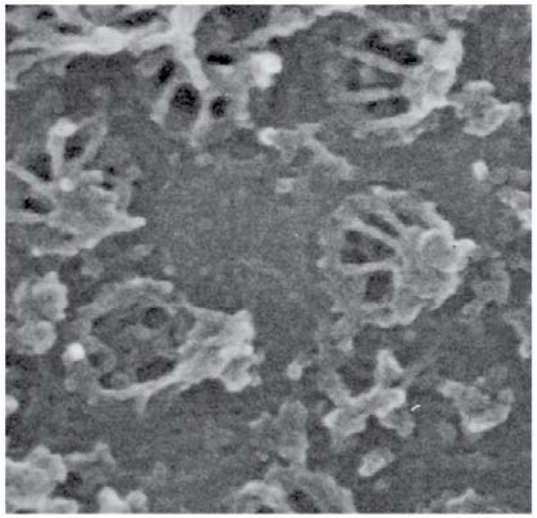




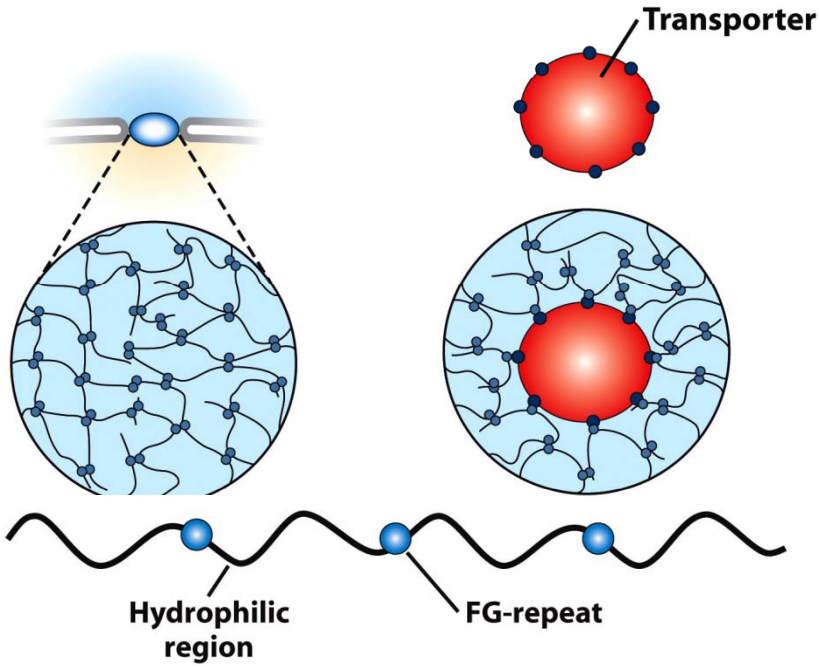
The nuclear pore: a molecular filter



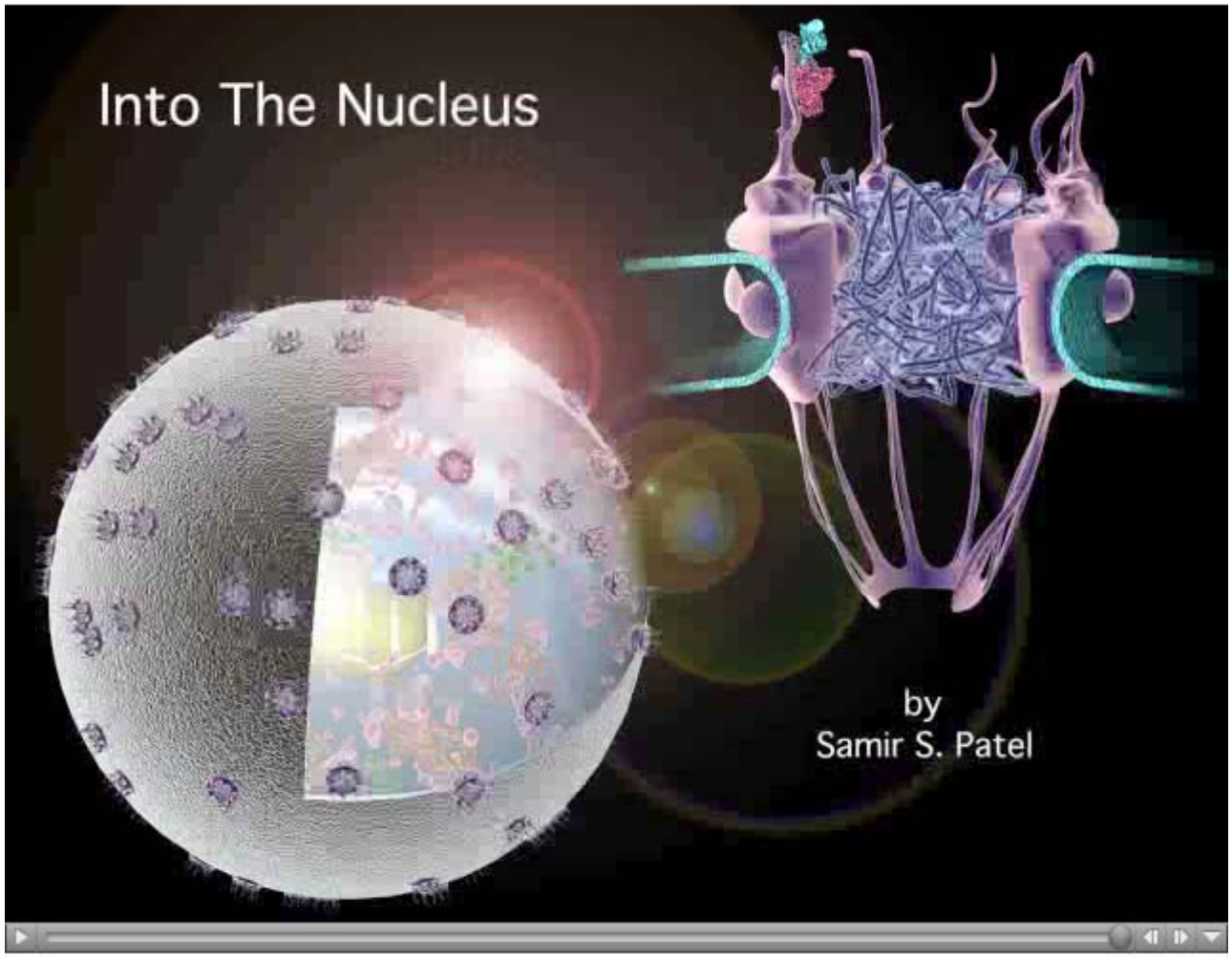




How the **molecular sieve** works:



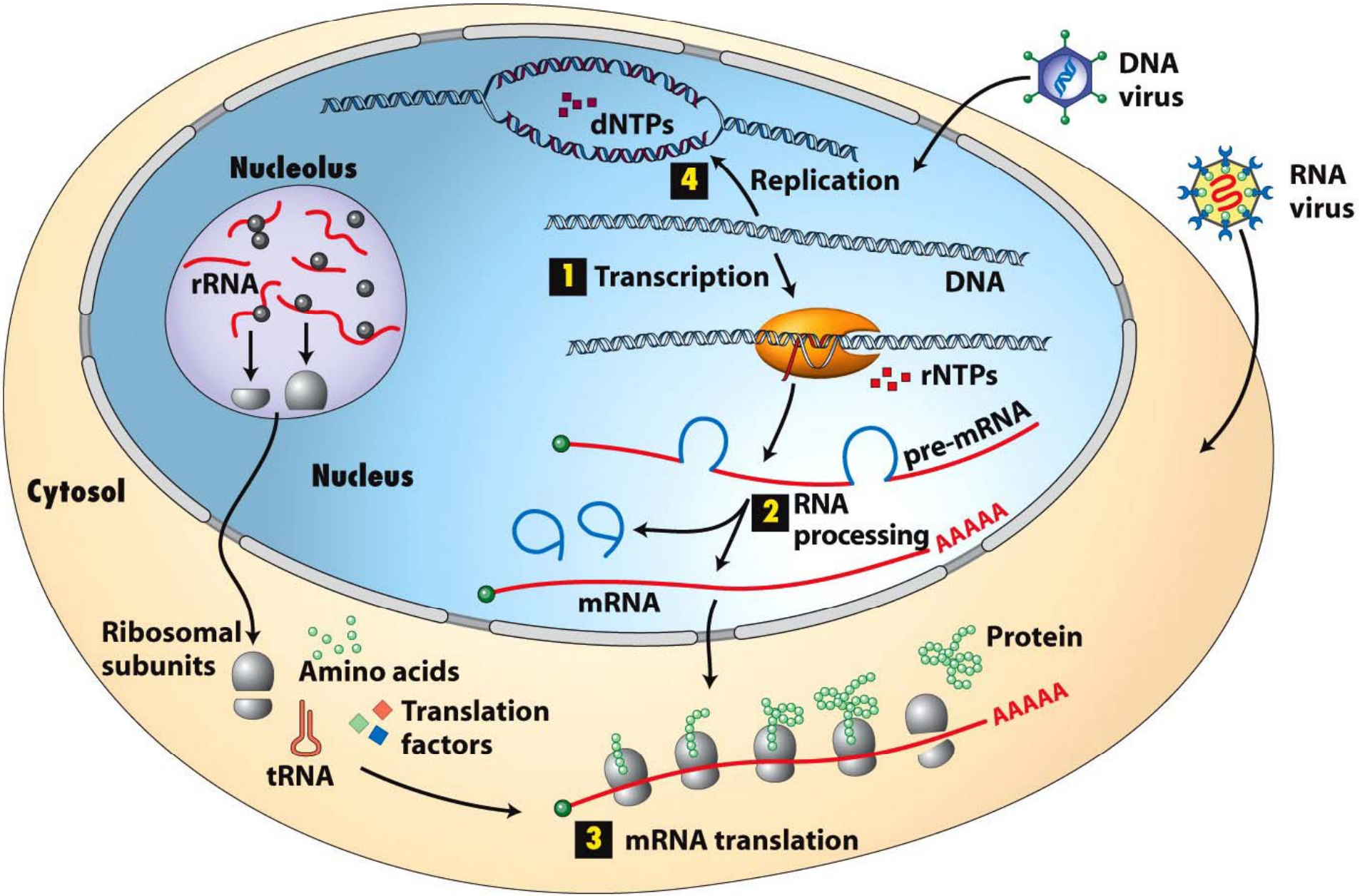
- The nuclear pore complex (**NPC**) is a complicated structure containing about 30 different proteins (nucleoporins)
- The **central channel** is filled with filamentous hydrophilic polypeptides
- The polypeptides contain **hydrophobic regions** (**FG-repeats** = Phenylalanine/Glycin)
- These structures are able to constantly and rapidly re-arrange **acting as a sieve** for small molecules
- A nuclear transporter can interact with the FG-repeats shuttling other molecules



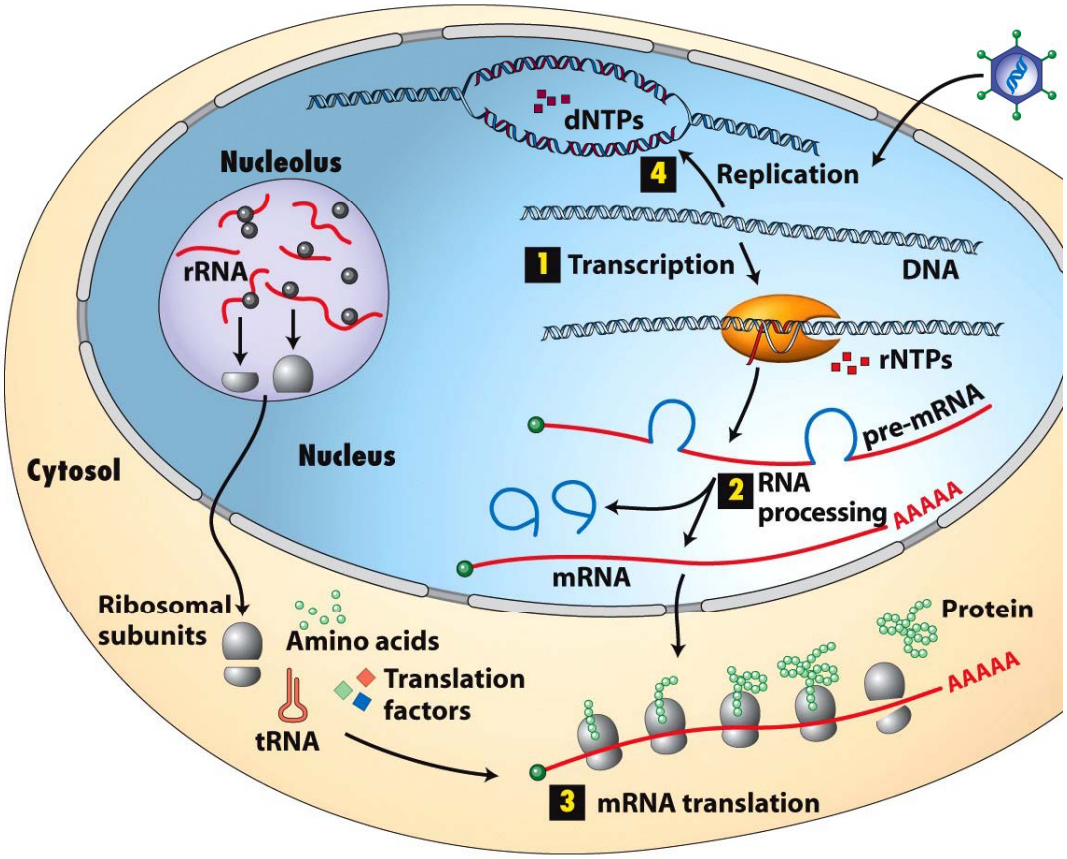
Animation
IntoTheNucleus.mov

http://sspatel.googlepages.com/nuclearporecomplex2

The protein nano-factory

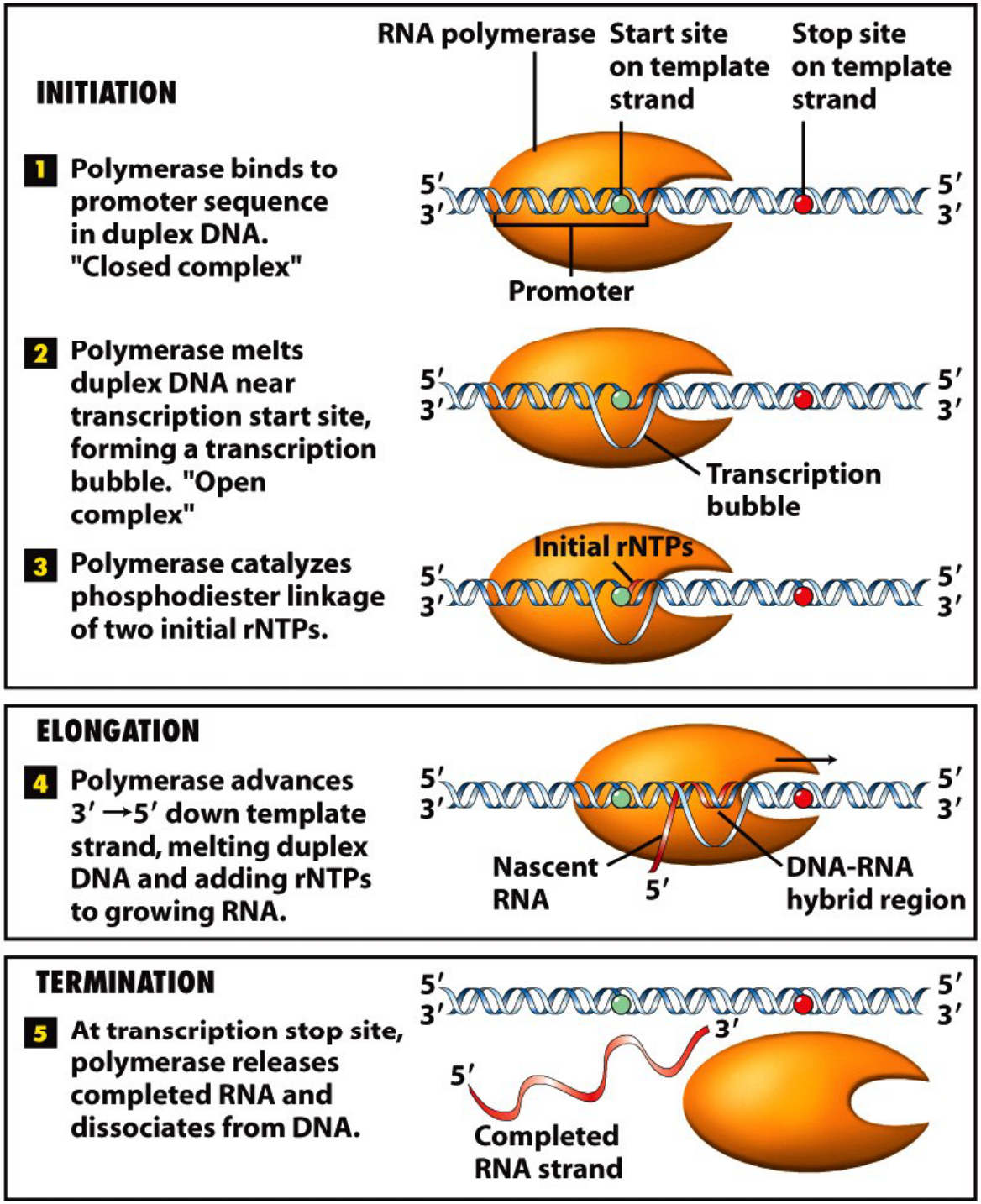


How to make a protein?



• **Construction plan** <u>of the proteins</u> is encoded in the **DNA**
• DNA is protected inside the nucleus
• Because proteins are made outside of the nucleus in the larger cytosolic space:
⇒ <u>Copy of DNA is made = mRNA</u>
⇒ Process is called **transcription**
• From mRNA code proteins are produced in the **ribosome-factory** = **translation**

DNA and RNA are both linear polymers composed of nucleotides (also called bases)

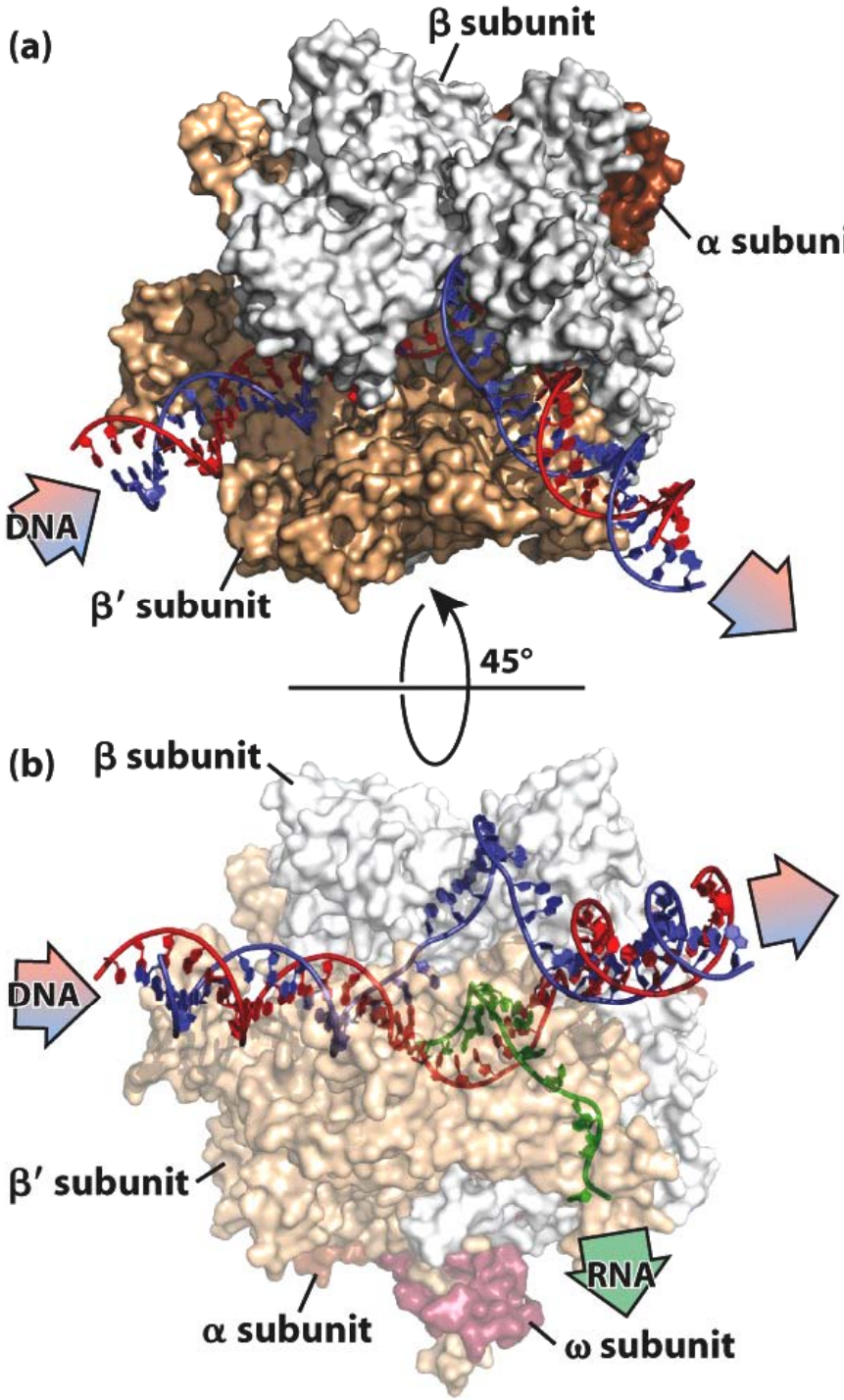


Transcription is powered by a <u>complicated molecular machine</u>:
The **RNA polymerase**

RNA polymerase is a <u>copy machine</u>:
It moves along the (double stranded) **DNA** and makes an exact (single stranded) copy (= **mRNA**)

3 steps:

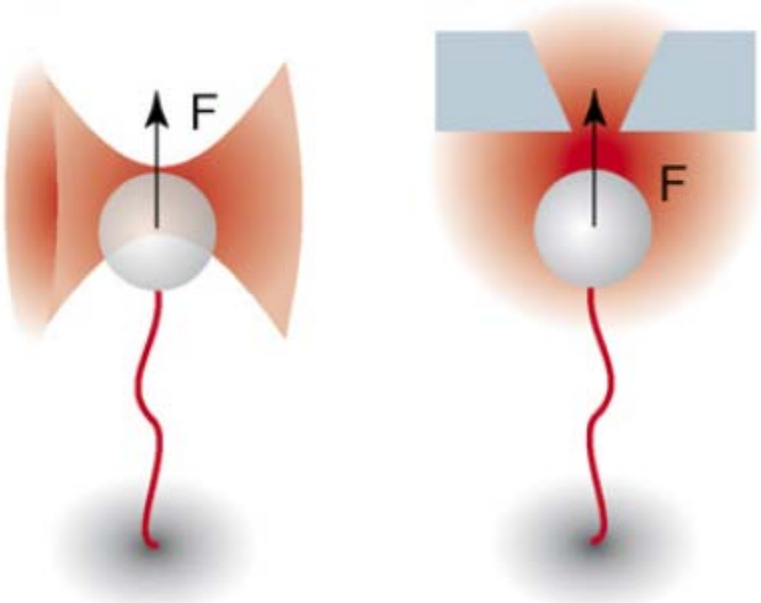
1) Complicated **initiation** step
2) **Elongation** (3' -> 5')
3) **Termination** (RNA released)



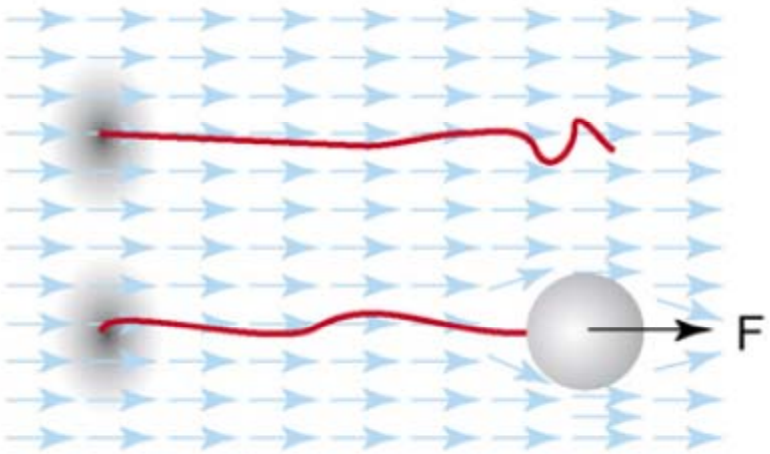
The RNA polymerase is macromolecular machine with a difficult design

- DNA is clamped between two subunits and the **double helix** is **opened**
- Then a **copy** from a single DNA strand is **made** into a single strand RNA

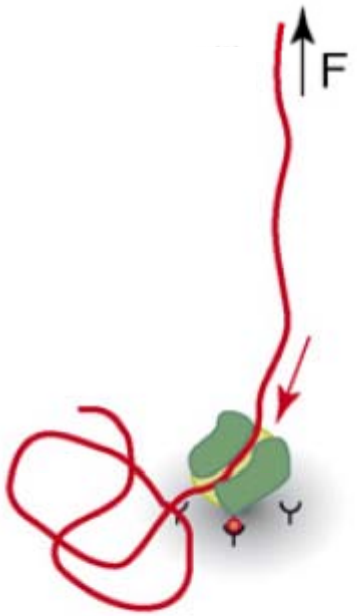
Single molecule methods to study DNA/RNA motors



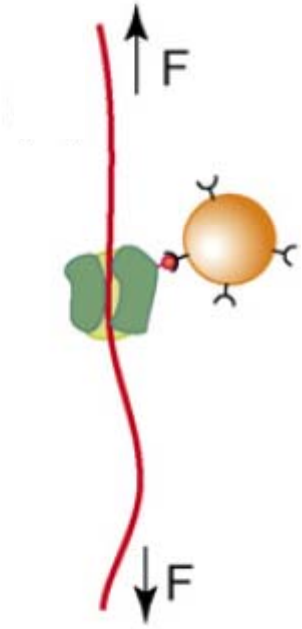
DNA or RNA is <u>first stretched by a bead</u> using **optical traps**, **magnetic beads** or **hydrodynamic flow**



Seidel and Dekker, 2007, Curr. Opin. Struct. Biol.

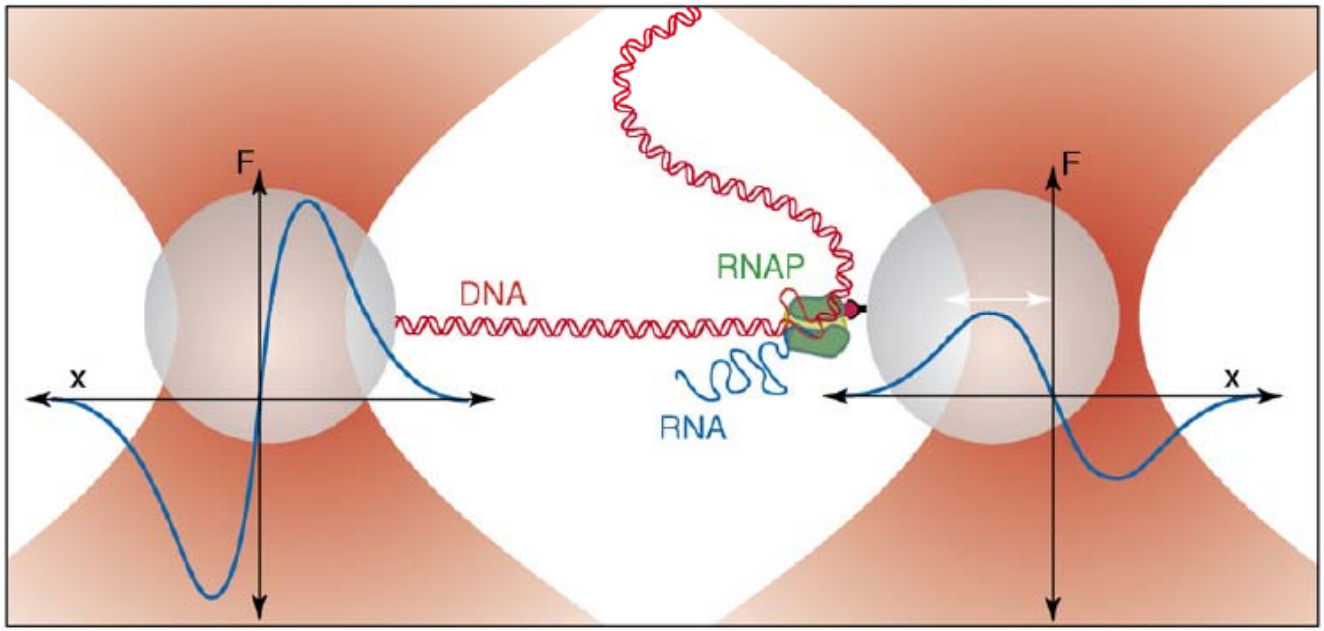


Immobilized DNA/RNA motor <u>shortens or lengthens</u> the DNA/RNA that can be detected by <u>bead displacements</u>

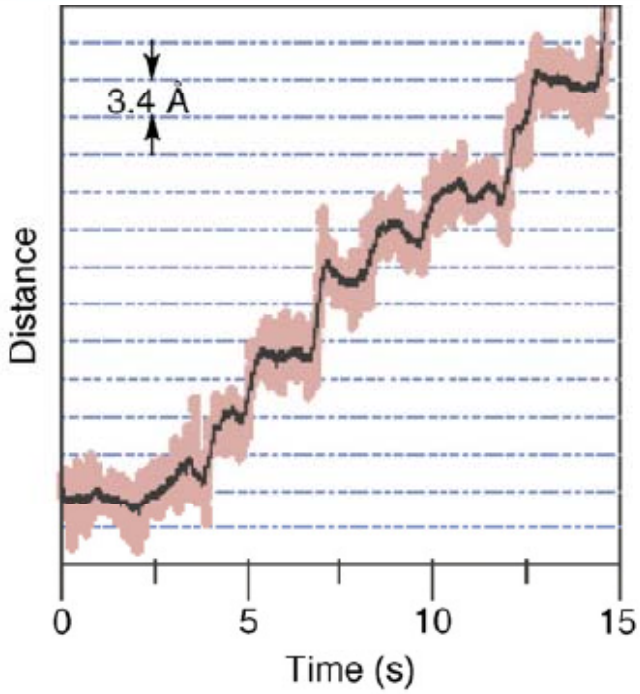


Direct motor movement on stretched DNA/RNA can be detected by <u>attaching a fluorescently labeled bead</u>

Detection of single base pair stepping by *E. coli* RNA polymerase



Two optical traps:
- One holds the DNA with **strong force**, the other holds the RNAP with **weak force**
- If RNAP moves, the attached bead is displaced (to the right)



Recorded single base pair steps of RNAP

Single molecule methods to study DNA/RNA motors

Properties of nucleic acid motors characterized using single-molecule techniques during the past two years[a].

Name	Type	Function	No. catalytic subunits	Velocity (bp s^{-1})[b]	Force (pN)[c]	Processivity (bp)[d]	Step size (bp)
E. coli RNA polymerase	RNA polymerase	Transcription	1	16	25	Several kbp	1
T7 RNA polymerase	RNA polymerase	Transcription	1	130	16	>1000	1
FtsK	dsDNA translocase	Chromosome segregation	6	5000	40	>5000	2 or 13
Φ29 portal motor	dsDNA translocase	Viral packaging	5	100	57	15 000	NN
RuvAB	dsDNA translocase	Migrates Holliday junctions	6	43	25	4000	NN
HCV NS3 RNA helicase	RNA helicase	HCV replication	1 or 2	50	NN	18	11
*Eco*R124I	dsDNA translocase	Type I restriction enzyme	1	550	>5	5000	1–2
RSC complex	dsDNA translocase	Chromatin remodeling	1	350	>2	400	12
Rad54	dsDNA translocase	Homologous recombination	NN	300	NN	12 000	NN
RecBCD	DNA helicase	dsDNA break processing	2	520	8	30 000	<6 or 23
B. subtilis DNA uptake	ssDNA translocase	Horizontal gene transfer	NN	80	45	>10 000	NN
T7 replisome	DNA replicase	DNA unwinding and synthesis	6 and 1	160	NN	17 000	NA

Seidel and Dekker, 2007, Curr. Opin. Struct. Biol.

TheScientist

December 2009 issue

FULL SPEED AHEAD

Physical forces acting in and around cells are fast—and making waves in the world of molecular biology. BY JEF AKST ILLUSTRATIONS BY ANDREW MEEHAN

When it comes to survival, few things are more important than being able to respond quickly to a change of circumstances. And when it comes to fast-acting indicators, it turns out that signals induced by physical forces acting in and around cells, appropriately dubbed biomechanical signals, are the champions of the cellular world.

"If you look at this mechanical signaling, it's about 30 meters per second—that's very fast," says bioengineer Ning Wang of the University of Illinois at Urbana-Champaign. That's faster than most family-owned speedboats, and second only to electrical (e.g., nerve) impulses in biological signaling. By comparison, small chemicals moving by diffusion average a mere 2 micrometers per second—a speed even the slowest row boater could easily top.

Indeed, when the two signal types were pitted against each other in a cellular race last year, the mechanical signals left chemical signals in their wake, activating proteins at distant sites in the cytoplasm in just a fraction of a second, at least 40 times faster than their growth factor opponent.[1] Mechanical signals are so fast, Wang adds, they are "beyond our resolution," meaning that current imaging techniques cannot capture the very first cellular changes that result from mechanical stress, which occur within nanoseconds.

For centuries, scientists have scrutinized the molecular inner workings of the body, with little or no regard to the physical environment in which these biological reactions take place. But the growing realization that physical forces have a pervasive presence in physiology (operating in a variety of bodily systems), and act with astonishing speed, has caused many to consider the pos-

" If you look at mechanical signaling, it is about **30 m/s**.

This is faster then any speed-boat and second only to electrical signaling (e.g., nerve).

By comparison, small chemicals move by diffusion only **2 µm/s** (compared to a very slow row boater).

Mechanical stimulation at one side of the cell can activate proteins at distant sites 40 times faster as for a growth factor would do.

It is now very difficult to measure the mechanical response on cell with common methods as they occur **within nanoseconds**."

In the late 1990s, however, closer examination revealed that the cell's interior is in fact a highly structured environment, composed of a network of filaments.[2] Pull on one side of the cell, and these filaments will transmit the force all the way to other side, tugging on and bumping into a variety of cellular structures along the way—similar to how a boat's wake sends a series of small waves lapping up on a distant and otherwise peaceful shoreline.

Mechanical signaling may be just as important as chemical communication in the life of a cell.

Scientists are now realizing the potential of such intracellular jostling to induce molecular changes throughout the cell, and the search for mechanosensing molecules has escalated dramatically in scope, including, for example, several proteins of the nucleus.

It's a search that will likely last a while, predicts cell biologist Donald Ingber, director of the Wyss Institute for Biologically Inspired Engineering at Harvard University. "To try to find out what's the mechanosensor is kind of crazy at this point," he says. As scientists are now learning, "the whole cell is the mechanosensor."

A key player, most agree, is the cytoskeleton, which is comprised of a variety of microfilaments, including rigid actin filaments and active myosin motors—the two principle components of muscle. Activation of the so-called nonmuscle myosins causes the cytoskeleton to contract, much like an arm muscle does when it lifts a heavy object.

The first intimation that the cytoskeleton could go beyond its established inner-cell duties (molecule transport and cell movement and division) came in 1997, when Ingber did the logical (in hindsight, at least) experiment of pulling on the cells to see what happened inside.[4] Using a tiny glass micropipette coated in ligands, Ingber and his team gently probed the surface proteins known as integrins, which secure the cell to the extracel-

< BONE

Given the ostensible inflexibility of bone, it may seem counterintuitive to imagine mechanical force playing a significant role in the skeletal system. But as every astronaut knows, bones are actually quite dynamic, and physical force (or lack thereof) can trigger changes that affect bone growth and strength. Astronauts, for example, experience significant bone degeneration after long stints in space, where their bodies are not exposed to the constant pull of gravity, and paraplegic patients lose between 25 and 30% of their bone mass within the first month of being paralyzed.

Despite the well-established response of bone to mechanical loading, however, the mechanism by which it senses such forces has been "an age-long mystery," says bioengineer Sheldon Weinbaum of the City College of New York. Because bone is so stiff, normal physiological stress rarely induces more than a 0.1% strain, meaning that bone is compressed just 1/10 of 1% of its length. Yet in vitro experiments on bone required strains of 1–3% to produce a cellular response—a force that would likely cause bone damage.

The answer came in the mid-1990s in the form of fluid flow. The calcified matrix of bone consists of cavities known as lacunae that are connected via a network of canals known as canaliculi, which carries interstitial fluid through the skeletal system. Originally proposed as a system for delivering nutrients and removing waste products from bone cells called osteocytes, scientists now recognize fluid flow through this lacuno-canalicular network as providing bone tissue with important mechanical loading information.

In 2001, Weinbaum and his colleagues suggested that "tethering" filaments strung between bone cells and the walls of the lacuno-canalicular network may act as a sensor—and amplifier—of physical forces.[10] Indeed, the drag forces inflicted on these tethers as the result of fluid flow can amplify a mechanical signal 10 to 100 times greater than a signal imposed directly on the bone matrix, but how this signal elicits a biochemical response is unclear. An alternative hypothesis arose in 2007, when Weinbaum and his colleagues identified integrin attachments on the canalicular wall. Their work suggested that these integrins—which transmit and receive mechanical forces via the cytoskeleton in other systems—may be the primary mechanotransducer in bone, resulting in intracellular signals two orders of magnitude greater than the strains of the bone itself.[11]

HOW MECHANICAL SIGNALS AMPLIFY IN BONE CELLS

As interstitial fluid flows through the networks of cavities and canals known as the lacuno-canalicular network, it pulls on "tethering" filaments that link osteocytes, or bone cells, and the walls of the canaliculi. These drag forces on filaments can then amplify and transmit mechanical forces to the osteocytes. Projections of the canaliculi wall, attached to the osteocyte at an integrin protein, may also participate in amplifying and transmitting the signal.

lular matrix. When they quickly pulled the micropipette away, they saw an immediate cellular makeover: cytoskeletal elements turned 90 degrees, the nucleus distorted, and the nucleolus—a small, dense structure within the nucleus that functions primarily in ribosome assembly—aligned itself with the direction of the applied force.

"That kind of blew people away," Ingber recalls. "It revealed that cells have incredible levels of structure not only in the cytoplasm but in the nucleus as well."

Wang (once a postdoc in Ingber's lab at the Harvard School of Public Health) and other collaborators combined a similar technique with fluorescent imaging technology to visualize how these forces were channeled within the cell's interior. Upping the resolution and further refining these techniques, Wang began mapping these intracellular forces as they made their way through the cell. In 2005, the maps confirmed the physical connection between the cell-surface integrins and the nucleus, and showed that these external forces follow a nonrandom path dictated by the tension of the cytoskeletal elements.[3]

The end point of these mechanical pathways is likely a mechanosensitive protein, which changes shape in response to the force,

"Biomechanics is becoming increasingly accepted, and people are recognizing its role in development, in disease, and in general cellular and tissue function."

–MOHAMMAD MOFRAD

BLOOD ›

Bioengineer John Tarbell of the City College of New York points to a small device that holds a matrix of dancing ink spots, lengthening and warping with the tug of the machine. "The stretch-and-shear device," he explains. "[In here], the cells get exposed to flow and to stretch." The spots, placed on an artificial membrane within the device's plastic walls, illustrate the effect of the machine's mechanical forces, to which Tarbell will eventually subject cell cultures and record the effects. It's like a drug-testing experiment, only instead of a drug, he and his team are exposing the cells to friction and stretching, two of the many mechanical forces cells lining blood vessels experience every day.

Recently, scientists have been gathering information showing how physical forces direct the development and restructuring of the cardiovascular system. Forces from blood flow can trigger blood vessels to dilate or contract. In particular, shear stress—the frictional force resulting from blood flow, which can range from just 1 pascal when an individual is resting to 10 pascals during heavy exercise—may initiate biochemical responses inside the cell that can affect such changes.

In 2005, researchers identified a transmembrane protein at cell-cell adhesions that connect endothelial cells to one another called PECAM1, which responds to stress by rapidly activating a Src family kinase.[7] This kinase appears to initiate downstream signaling pathways, including those involving integrins on the basal membrane of the cell. This activation is likely triggered by a conformational change in PECAM1 or other proteins, but "the understanding of those physical mechanisms isn't very good," says cell biologist Martin Schwartz of the University of Virginia.

To reach this lateral site of mechanotransduction, the shear forces are transmitted through cytoskeletal elements that link the membrane exposed to the flow to the cell-to-cell adhesions. Recently, work by Tarbell and others has suggested that the forces are propagated across the membrane through a dense layer of macromolecules that lines the surface, known as the glycocalyx. Compromising the glycocalyx, however, does not completely abolish the cell's response to physical force, suggesting that other membrane proteins play key roles, as well.

Most recently, scientists have recognized a role for shear stress in early development. Two studies published this past summer demonstrated that the initiation of the heartbeat and the first pulses of blood flowing through the young aorta spur the development of hematopoietic stem cell (HSC) production.[8,9] These findings suggest that the physical forces exerted by blood play a lifelong role in the physiology of the vascular system.

PHYSICAL FORCES IN ENDOTHELIAL CELLS

Blood flowing through the vascular system inflicts shear stress on the endothelial cells of the blood vessels. The force is transmitted through cytoskeletal elements to the cell-to-cell adhesions, where a transmembrane protein known as PECAM1 responds by activating downstream signaling pathways, including those involving a Src family kinase and integrins on the basal membrane of the cell. A dense layer of macromolecules that lines the surface, known as the glycocalyx, may participate in transmitting the force across the cell membrane.

Because Thompson "couldn't measure [the forces] at that time, that kind of thinking got pushed to the wayside as genetic thinking took over biology," says bioengineer Christopher Chen of the University of Pennsylvania. That is, until 2003, when Emmanuel Farge of the Curie Institute in France squeezed *Drosophila* embryos to mimic the compression experienced during early development and activated *twist*—a critical gene in the formation of the digestive tract.[4] These results gave weight to Thompson's idea that stress in the embryo stimulates development and growth, and inspired developmental scientists to begin considering mechanical effects, Chen says. "Now we're at the stage where there's a lot of interest and willingness to consider the fact that mechanical forces are not only shaping the embryo, but are linked to the differentiation programs that are going on."

> "To try to find out what's the mechanosensor is kind of crazy at this point. As scientists are now learning, the whole cell is the mechanosensor."
>
> -DONALD INGBER

Again, the cytoskeleton is a key player in this process. In fruit flies and frogs, for example, nonmuscle myosins contract the actin filaments to generate the compressive forces necessary for successful gastrulation—the first major shape-changing event of development. Myosins similarly influence proliferation in the development of the *Drosophila* egg chamber, with increased myosin activity resulting in increased cell division.

Cytoskeleton contractility also appears to direct stem cell differentiation. In 2006, Dennis Discher of the University of Pennsylvania demonstrated that the tension of the substrate on which cells are grown in culture is important for determining what type of tissue the cells will form.[5] Cells grown on soft matrices that mimic brain tissue tended to grow into neural cells, while cells grown on stiffer matrices grew into muscle cell precursors, and hard matrices yielded bone. In this case, it seems that stiffer substrates increased the expression of nonmuscle myosin, generating greater tension in the actin cytoskeleton and affecting differentiation. (Altering or inhibiting myosin contraction can also affect differentiation.)

More recently, in October, Wang induced changes in mouse embryonic stem (mES) cells by simply probing the cell surface.[6] Almost immediately after applying a small force to a surface integrin, each cell began spreading across the substrate—a key process in morphogenesis and germ layer formation. Tugging on the cells also down-regulated *oct3/4* expression—a sign of cell differentiation—further supporting a role for external forces in embryogenesis.

Developing specific cell types for clinical uses hinges on a more complete understanding of how cell fate is shaped in vivo, and the recognition that the physical environment plays a role in this process has "had a big effect on extending the importance of mechanics," Chen says. "There's always a good mechanical aspect of these biological problems," Mofrad adds. "[As] this is becoming increasingly evident, mechanics is taking a more prominent role." ■

Have a comment? E-mail us at mail@the-scientist.com

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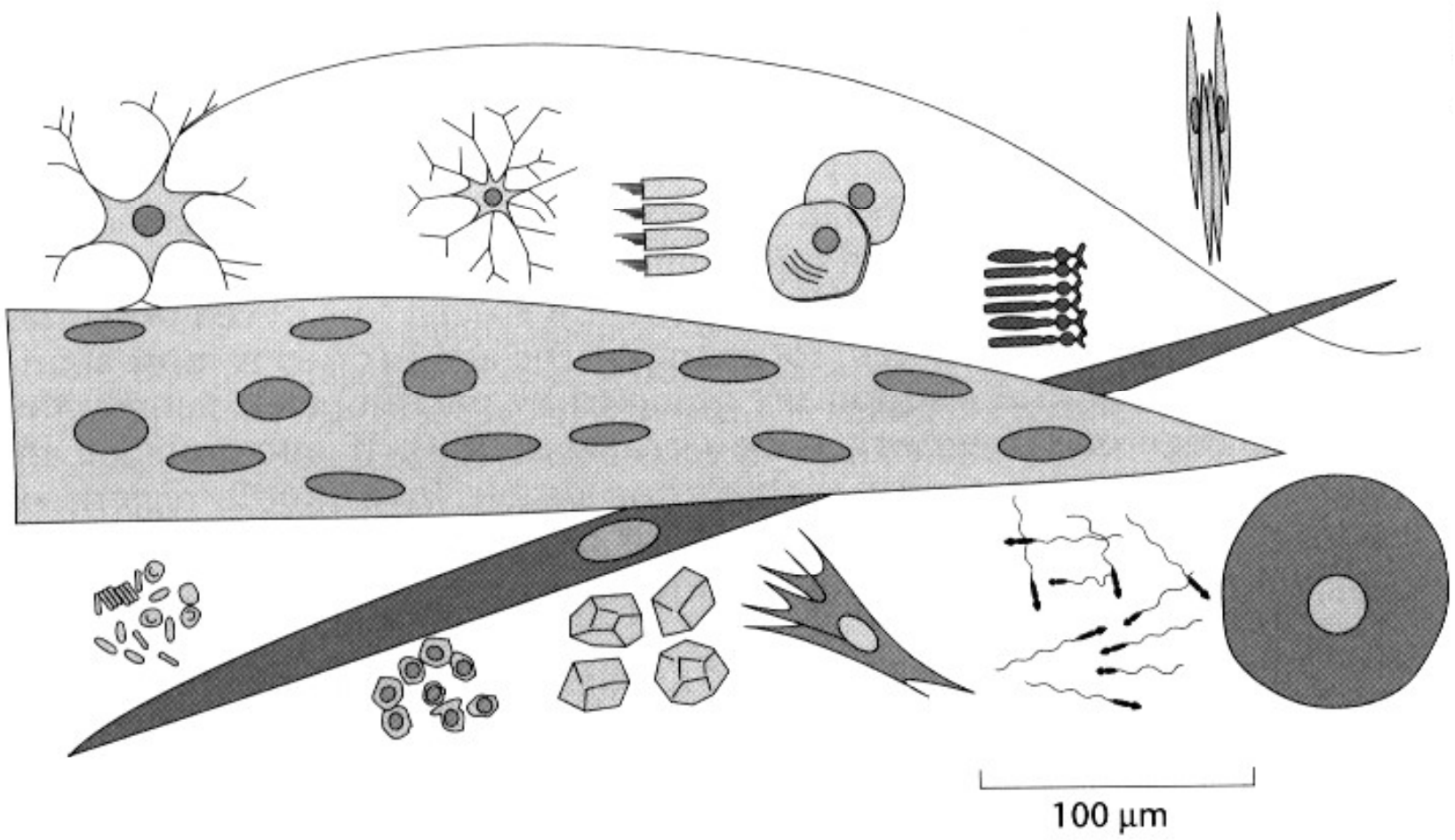
Cell mechanics: physical forces that maintain cell shape



Muscle cells, fibroblast, red blood cells, neurons, egg, sperm, hair cell, retinal cells...
... drawn to same scale.

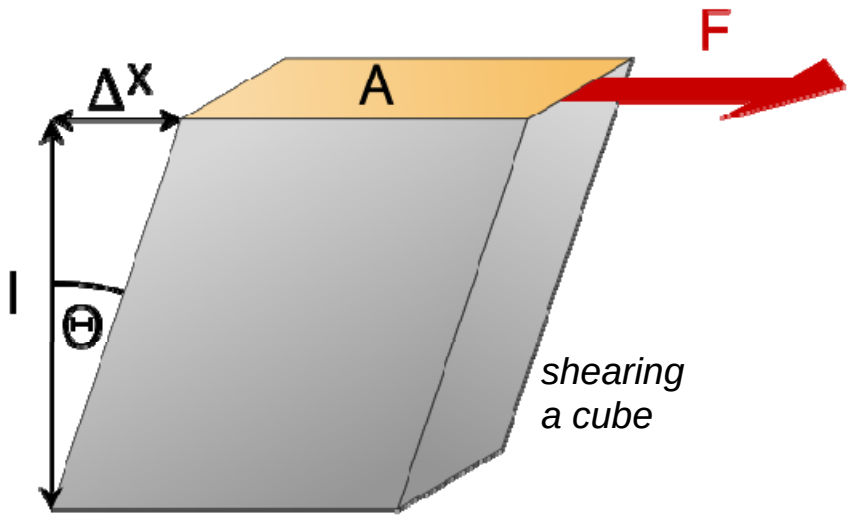
Bray, Cell Movements, 2nd Ed.

The quantities of cell mechanics

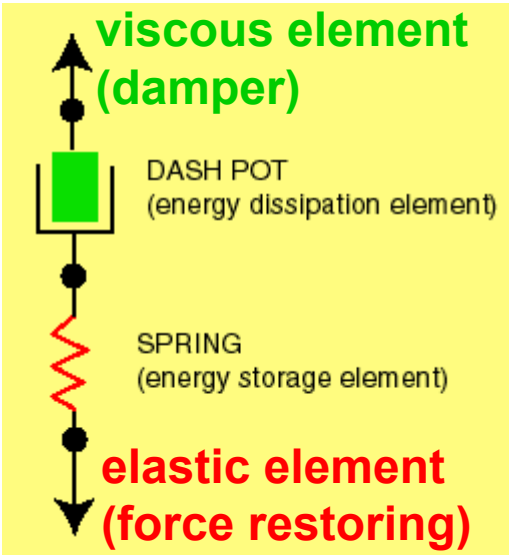
Cells have both, viscous and elastic properties, they behave **viscoelastic**

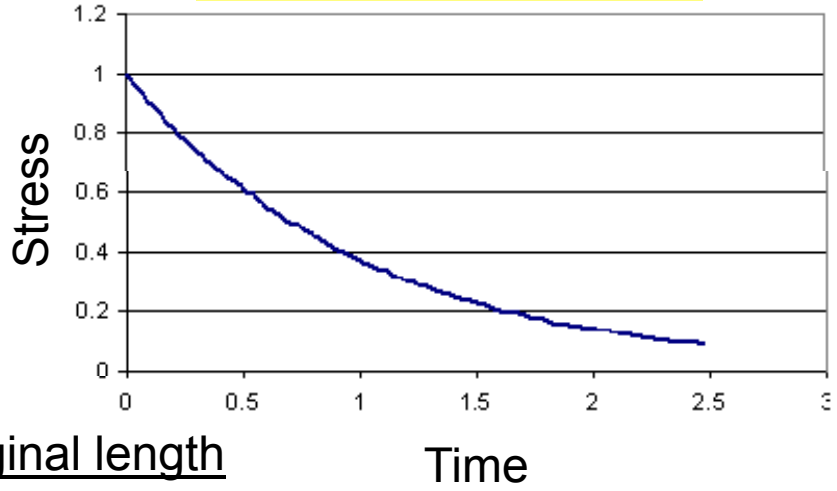
Shear stress δ = **F**orce per **A**rea or: $\sigma = \frac{F}{A}$ [Pa]

Strain γ = Deformation = $\Delta x/x_0$



Maxwell model of viscoelastic materials

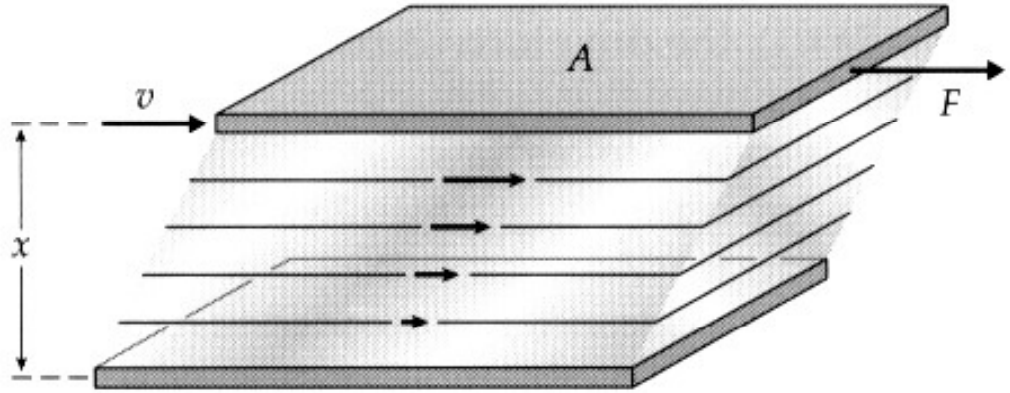




• If a Maxwell material is <u>suddenly strained</u> (deformed): <u>stresses decay with time</u>
• If we <u>suddenly free</u> the deformation:
elastic element = <u>spring back</u>
viscous element = <u>does not return to its original length</u>
=> Problem: **irreversible deformation component**

Anatomy of the viscous dashpot: viscous damping

Sheared liquid



Velocity gradient

Shear stress proportional to velocity gradient:

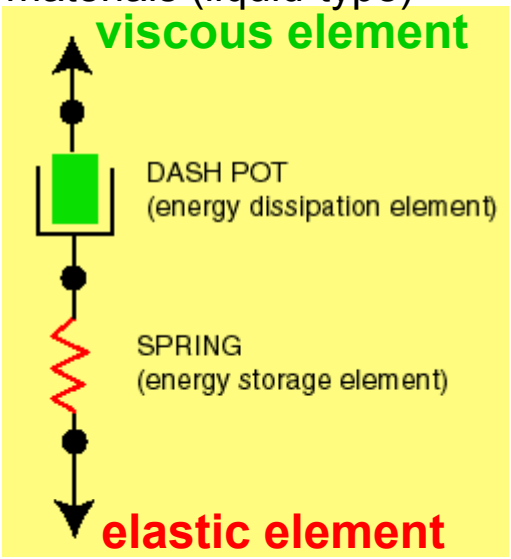
$$\frac{F}{A} = \eta \frac{dv}{dx}$$

- When a fluid is placed between to plates and the upper plate is moved while the lower plate is stationary a **velocity gradient** is observed
- The shear stress (F/A) is proportional to this velocity gradient (dv/dx)
- The **constant** η (êta) of this relation **is called** the **coefficient of viscosity**
- Because the unit for shear stress is Pa and the unit for the velocity gradient (= shear rate) is s^{-1}, the unit for the viscosity is Pa · s

The quantities of cell mechanics

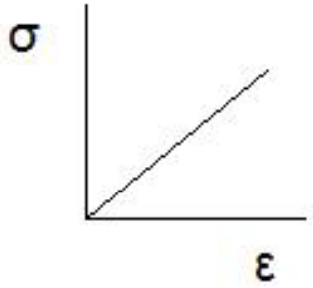
Problems of the Maxwell model

Maxwell model of viscoelastic materials (liquid-type)

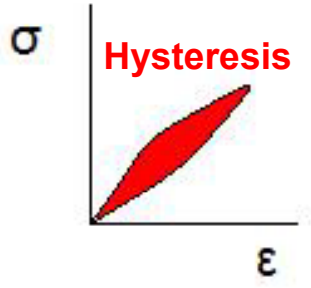


• If a Maxwell material is **suddenly stressed**:
elastic element = suddenly deform
viscous element = deform with a constant rate
• If material is **suddenly released** from stress:
elastic element: spring-back to its original value
viscous element: no change in deformation
• Further problem: Maxwell model not ideal for predicting **creep behavior** (because it describes the strain relationship with time as linear)

Stress/Strain curves



Elastic material



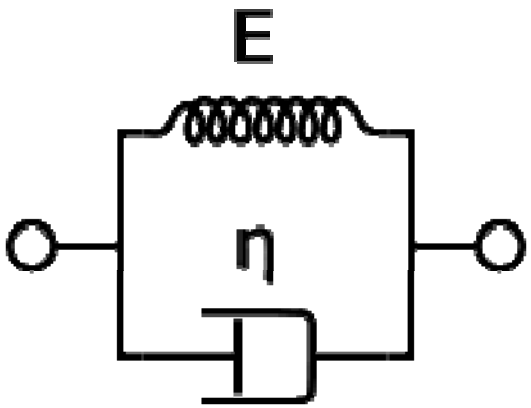
Viscoelastic material

Creep is the tendency of a solid material to slowly move or deform permanently under the influence of stresses

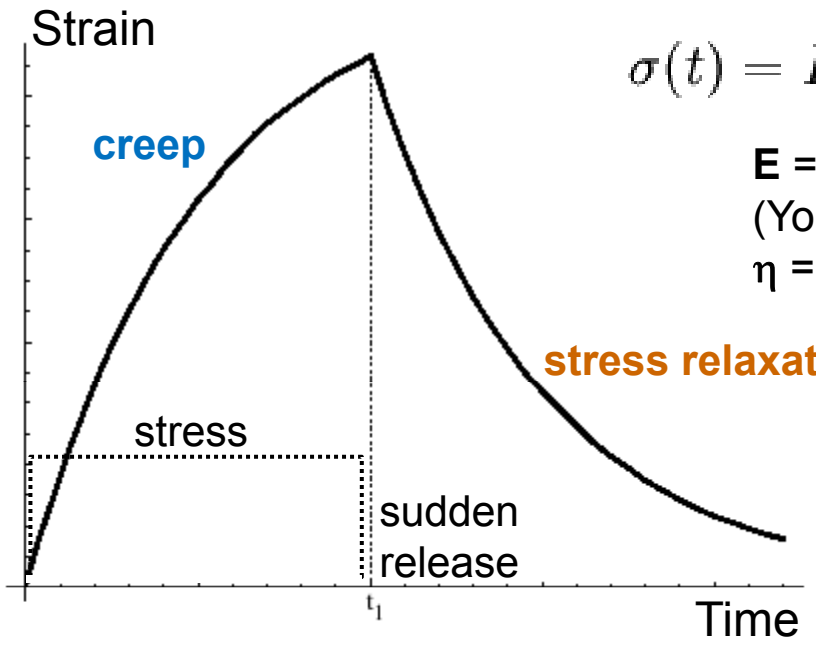
The quantities of cell mechanics

Kelvin-Voigt model describes well the creep behavior of viscoelastic materials

Kelvin-Voigt model of viscoelastic materials (solid-type)



Spring and dashpot in parallel



$$\sigma(t) = E\epsilon(t) + \eta\frac{d\epsilon(t)}{dt}$$

E = elastic modulus (Youngs modulus)
η = viscous modulus

- If we suddenly free the material from strain:

elastic element retard the material back until the deformation become zero

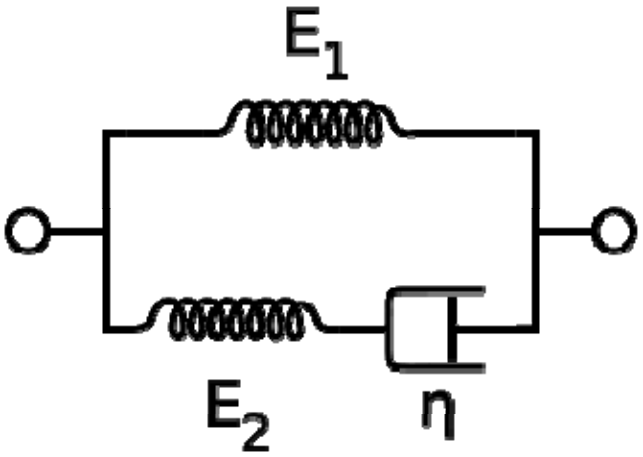
⇒ **elastic element** ***resets*** **dash-pot** = deformation is reversible

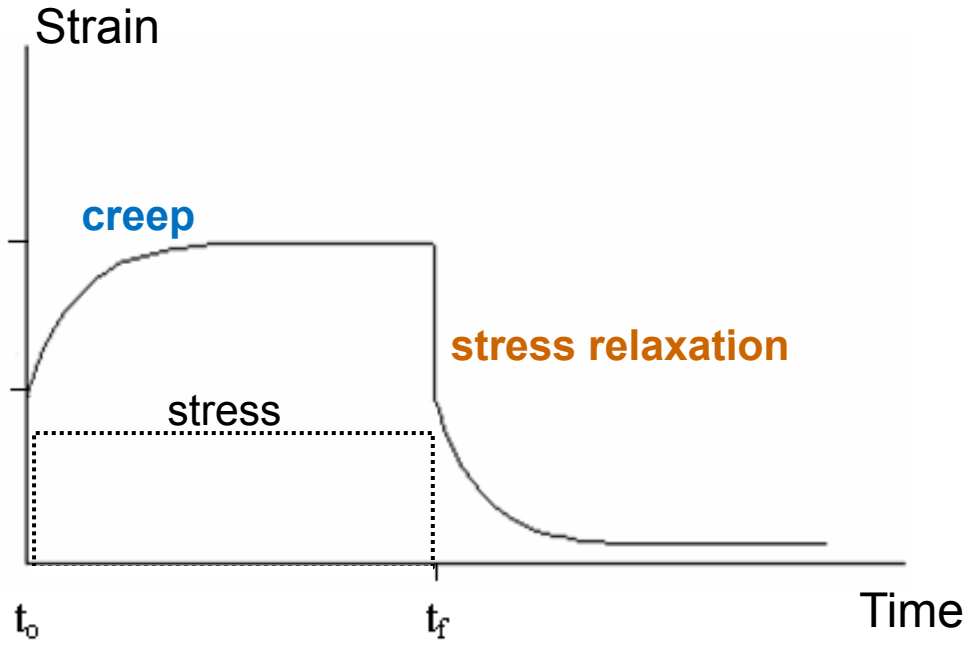
- Further: model better for describing **creep** behavior
- Problem: model not good to describe **stress relaxation** (here too continuous)

The quantities of cell mechanics

SLS model describes well the creep and stress relaxation of viscoelastic materials

Standard-Linear-Solid (SLS) model of viscoelastic materials



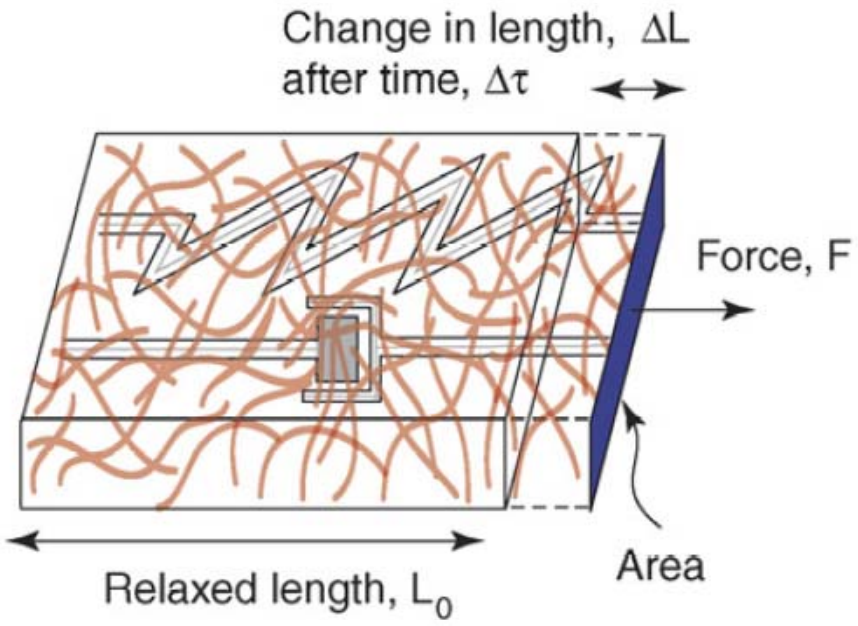


SLS model describes well **creep** and (discontinuous) **stress relaxation**

Is the cell a solid or a liquid?

The quantities of cell mechanics

Storage and loss modulus describing elastic and viscous behavior of cells



Stress: $\sigma = F/\text{Area}$

Strain: $\gamma = \Delta L / L_0$

Strain rate: $\dot{\gamma} = \Delta\gamma / \Delta\tau$ (temporal derivative of strain)

Elastic response: $\sigma = G' \gamma$

Viscous response: $\sigma = [\, G''(\Delta\tau)\, \Delta\tau \,]\, \dot{\gamma}$

- **Elasticity** of **biopolymer** networks allows them to resist deformation **like a spring**

⇒ energy of deformation is stored regardless of time: **storage modulus G´**

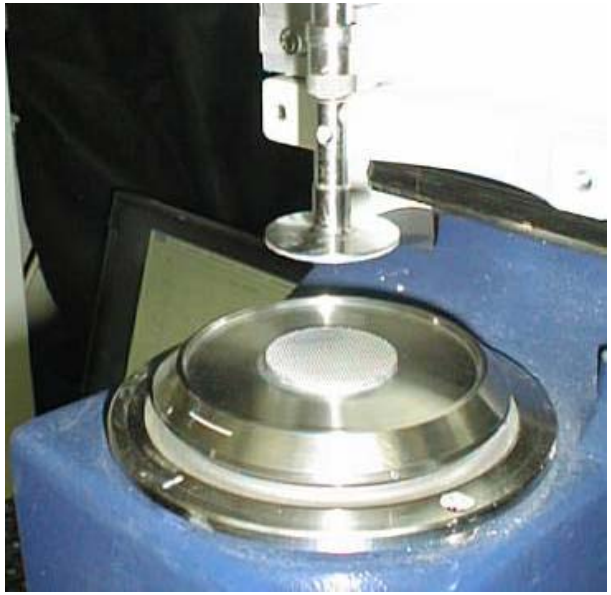
- **Viscous behavior** of **biopolymer** networks allows them to **flow as a fluid**:

⇒ resistance depends on the rate of deformation (like in a dashpot)

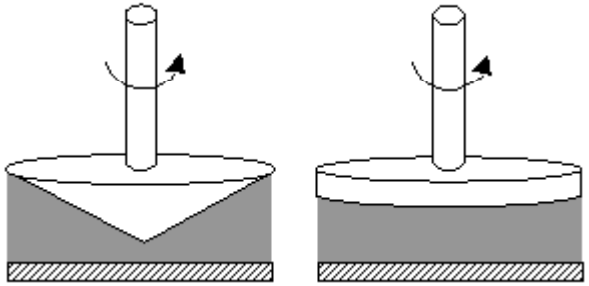
⇒ energy put into deformation: dissipated or lost: **loss modulus G´´**

Rheology: determination of viscoelastic properties of liquids

- Rheo = flow (Greek) = measuring the flow of liquids
- Most popular: cone-plate or plate-plate **rheometer** = liquid placed between 2 plates
- Upper plate rotates at defined speed and angle = **shear rate** (velocity per distance)
- Upper plate also measures the resistance (response) of the fluid to applied shear by measuring the **torque** (= twisting force) = **shear stress** (F/A)



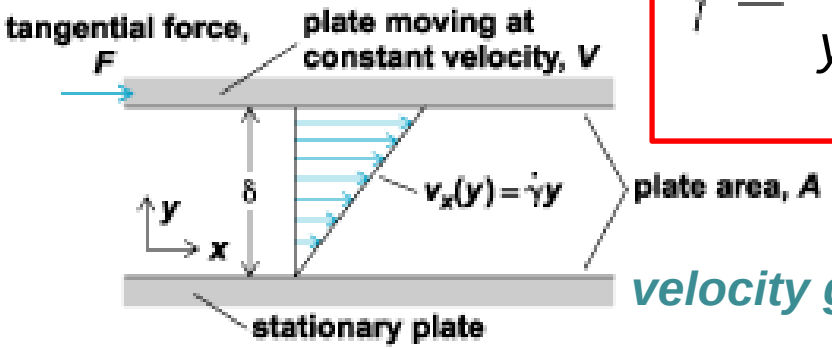
Oscillating cone or plate



Fixed plate

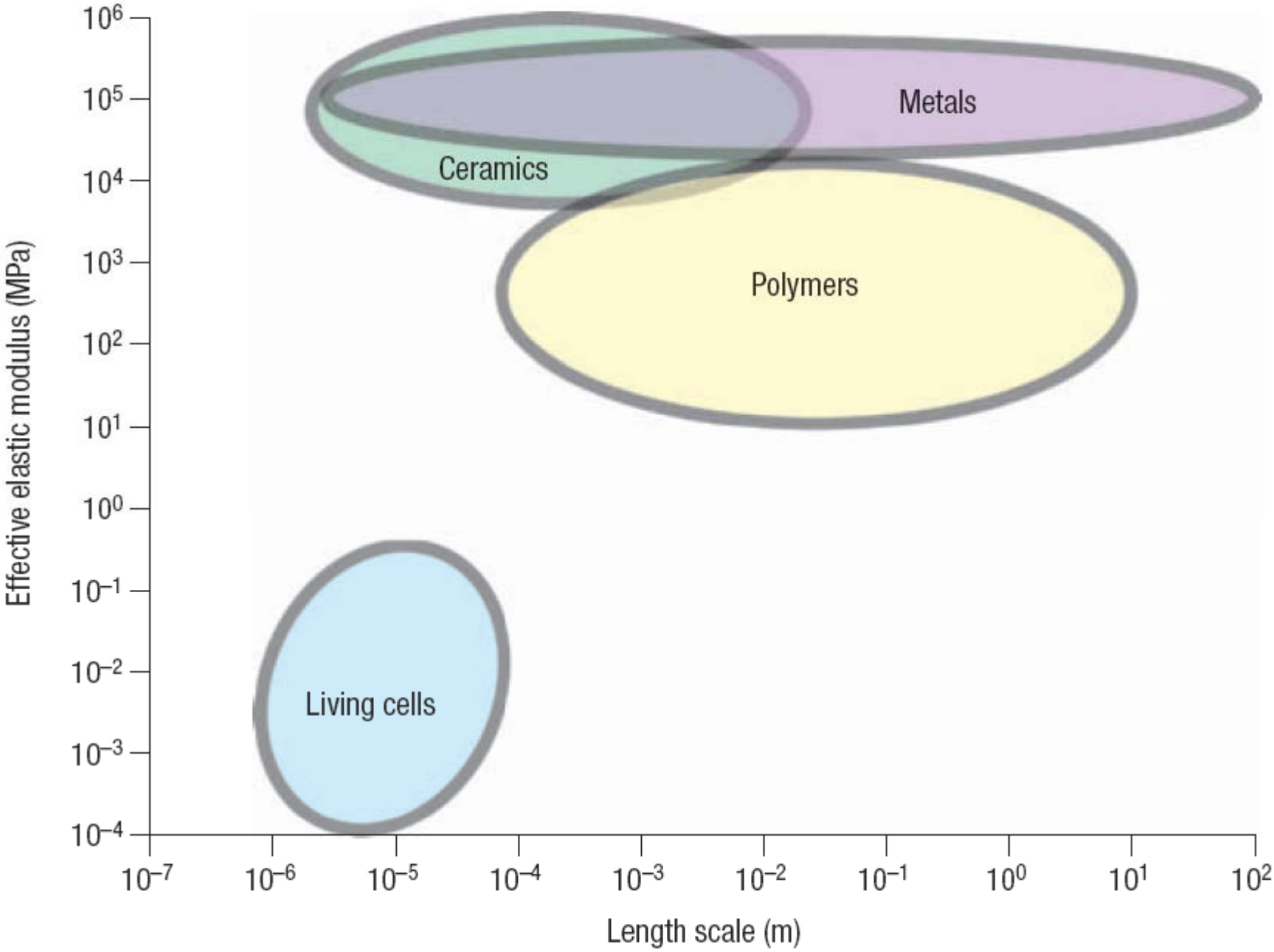
$$\dot{\gamma} = \frac{v}{y}$$

Shear rate: velocity per distance (distances between plates)



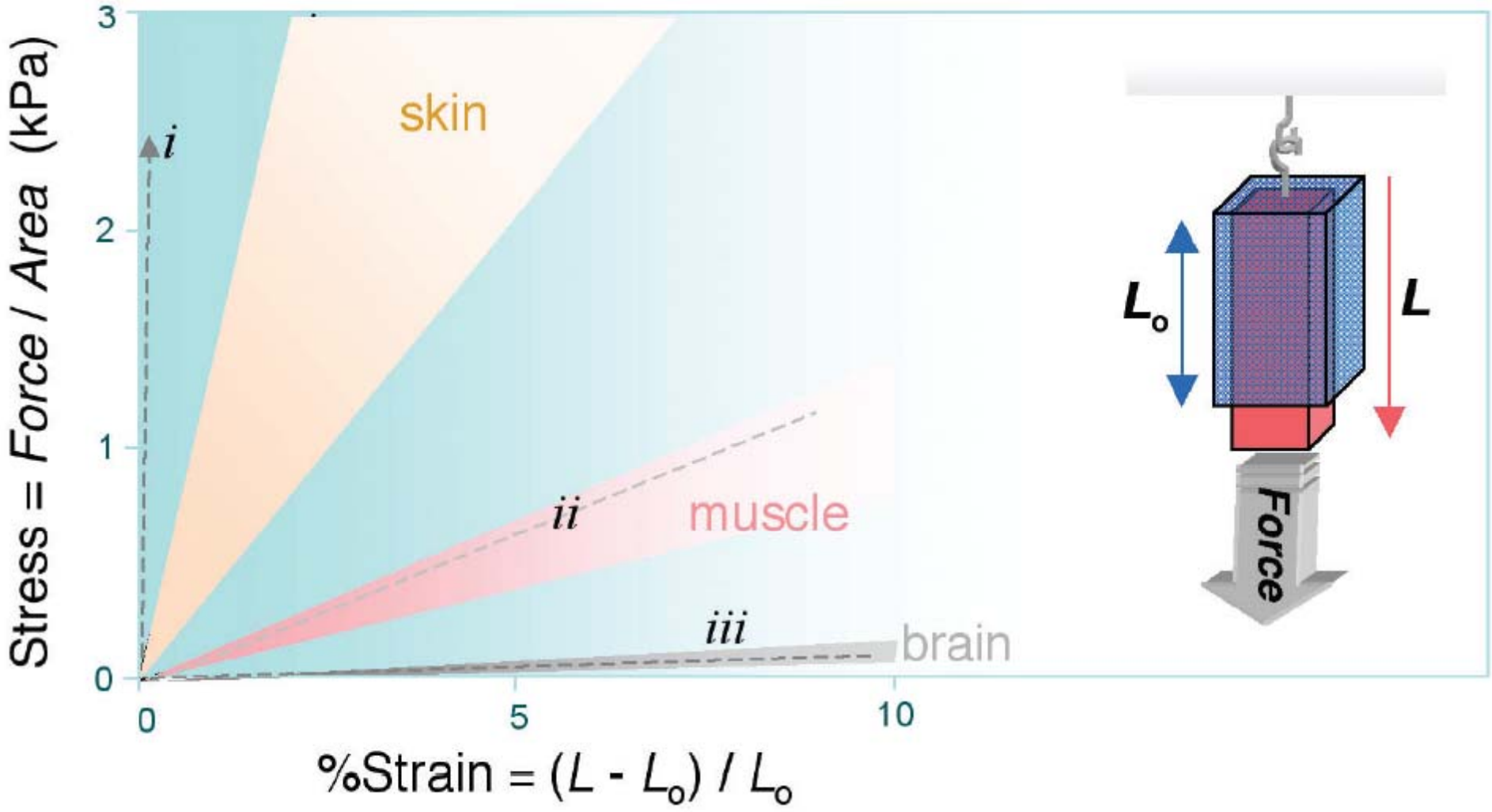
velocity gradient

Range of elastic moduli of cells compared with metals, ceramics and polymers



Strain/stress plot for different tissues

- To stretch (strain) **skin tissue**, a considerable amount of force (stress) is needed
- **Muscle tissues** can be deformed (strain) easily using only low forces (stress)
- **Brain tissue** does not show any elastic behavior (negligible strain/stress features)



Dischер et al., Science, 2005

Methods to measure the mechanical properties of cells