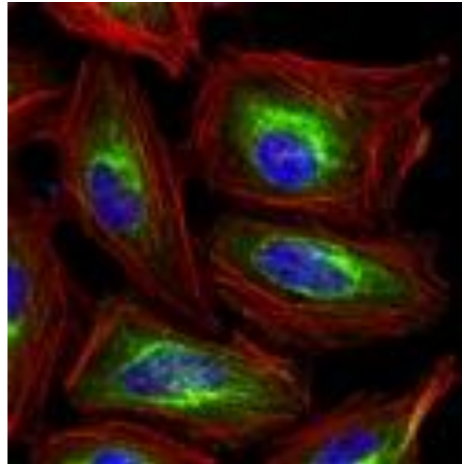
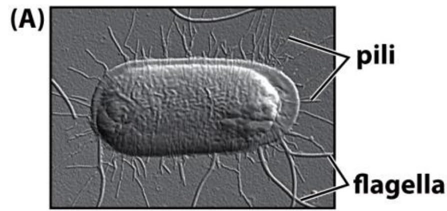
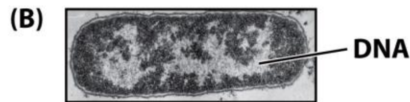
Quantitative Methods for Biological Systems

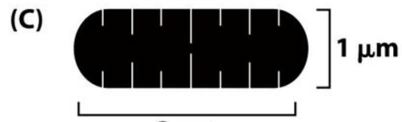
Lecture 02

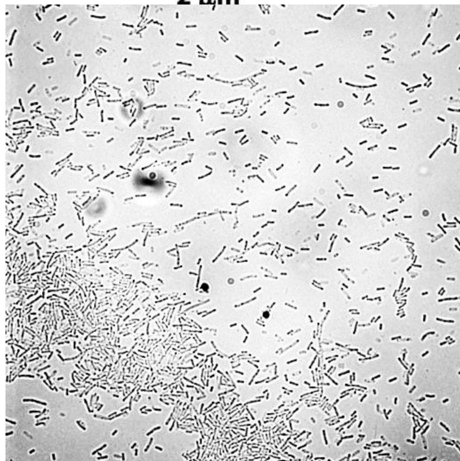


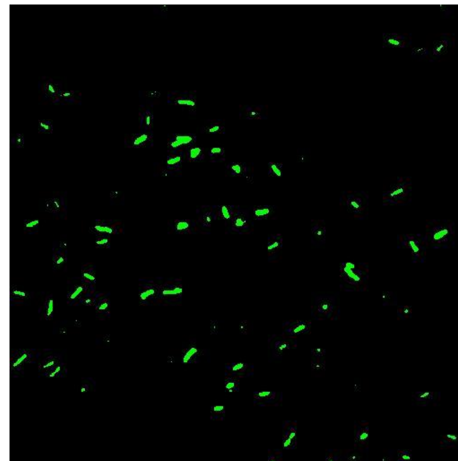
E coli has a machinery inside to produce $4x10^6$ proteins in less than 30 minutes

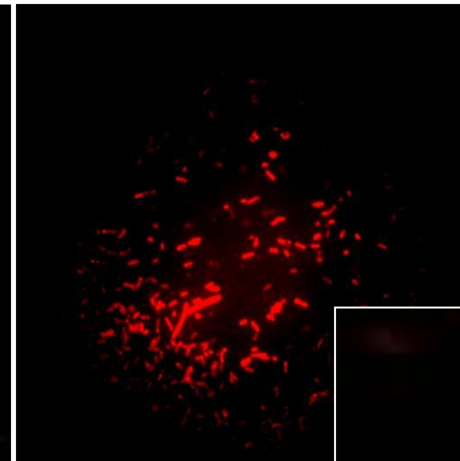


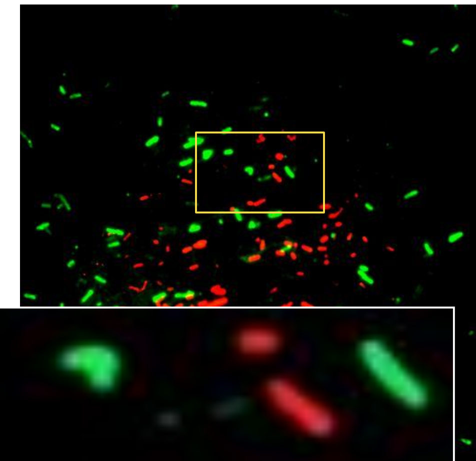






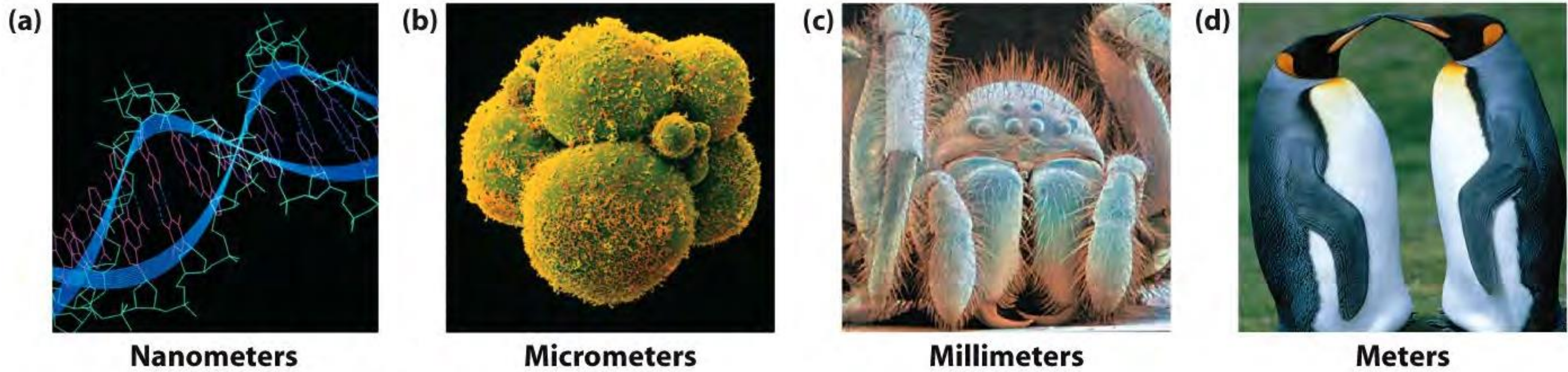






How you model the shape of the e.coli?

Cellular Size Scales



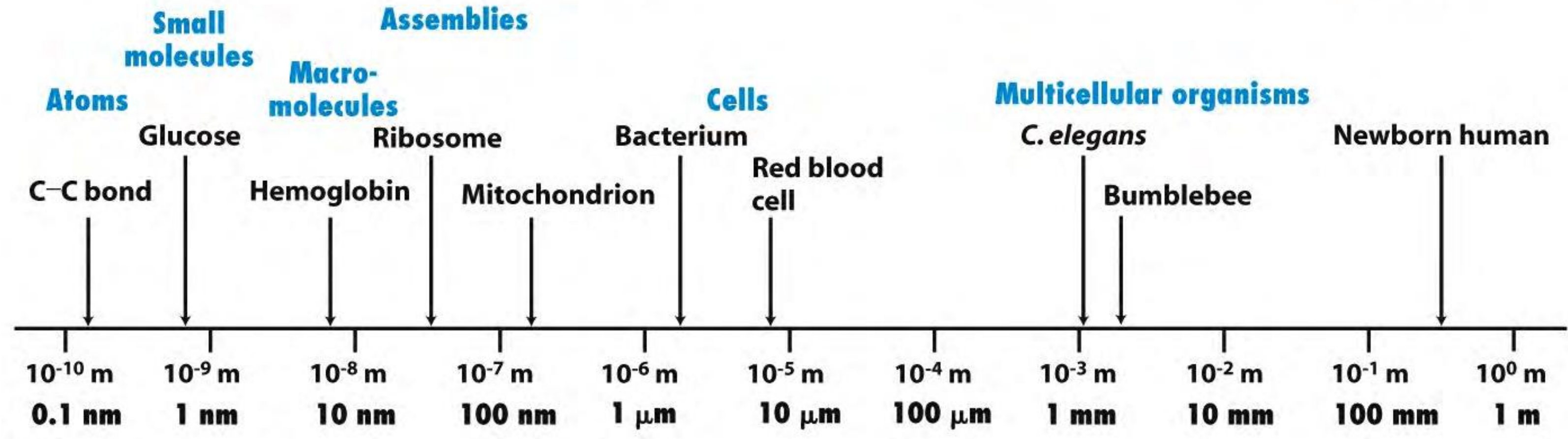


Table 1.1 Rules of thumb for biological estimates

	Quantity of interest	Symbol	Rule of thumb
E. coli	Cell volume	$V_{E.\ coli}$	$\approx 1\ \mu m^3$
	Cell mass	$m_{E.\ coli}$	≈ 1 pg
	Cell cycle time	$t_{E.\ coli}$	≈ 3000 s
	Cell surface area	$A_{E.\ coli}$	$\approx 6\ \mu m^2$
	Genome length	$N_{bp}^{E.\ coli}$	$\approx 5 \times 10^6$ bp
	Swimming speed	$v_{E.\ coli}$	$\approx 20\ \mu m/s$
Yeast	Volume of cell	V_{yeast}	$\approx 60\ \mu m^3$
	Mass of cell	m_{yeast}	≈ 60 pg
	Diameter of cell	d_{yeast}	$\approx 5\ \mu m$
	Cell cycle time	t_{yeast}	≈ 200 min
	Genome length	N_{bp}^{yeast}	$\approx 10^7$ bp
Organelles	Diameter of nucleus	$d_{nucleus}$	$\approx 5\ \mu m$
	Length of mitochondrion	l_{mito}	$\approx 2\ \mu m$
	Diameter of transport vesicles	$d_{vesicle}$	≈ 50 nm
Water	Volume of molecule	V_{H_2O}	$\approx 10^{-2}\ nm^3$
	Density of water	ρ	$1\ g/cm^3$
	Viscosity of water	η	≈ 1 centipoise (10^{-2} g/(cm s))
	Hydrophobic embedding energy	$\approx E_{hydr}$	25 cal/(mol Å^2)
DNA	Length per base pair	l_{bp}	$\approx 1/3$ nm
	Volume per base pair	V_{bp}	$\approx 1\ nm^3$
	Charge density	λ_{DNA}	2 e/0.34 nm
	Persistence length	ξ_P	50 nm
Amino acids and proteins	Radius of "average" protein	$r_{protein}$	≈ 2 nm
	Volume of "average" protein	$V_{protein}$	$\approx 25\ nm^3$
	Mass of "average" amino acid	M_{aa}	≈ 100 Da
	Mass of "average" protein	$M_{protein}$	$\approx 30{,}000$ Da
	Protein concentration in cytoplasm	$c_{protein}$	≈ 300 mg/mL
	Characteristic force of protein motor	F_{motor}	≈ 5 pN
	Characteristic speed of protein motor	v_{motor}	≈ 200 nm/s
	Diffusion constant of "average" protein	$D_{protein}$	$\approx 100\ \mu m^2/s$
Lipid bilayers	Thickness of lipid bilayer	d	≈ 5 nm
	Area per molecule	A_{lipid}	$\approx \frac{1}{2}\ nm^2$
	Mass of lipid molecule	m_{lipid}	≈ 800 Da

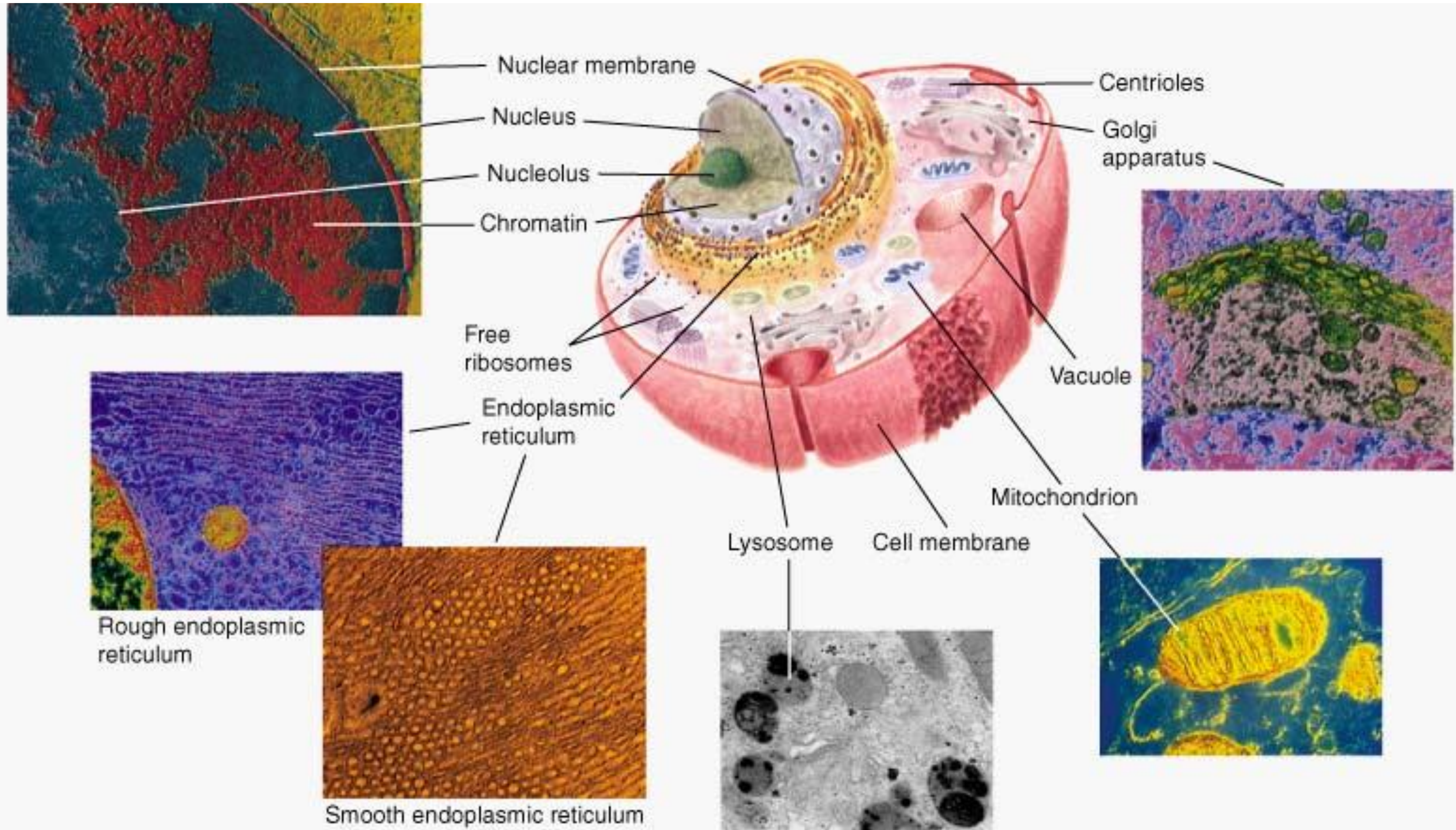
Table 1.1 Physical Biology of the Cell (© Garland Science 2009)

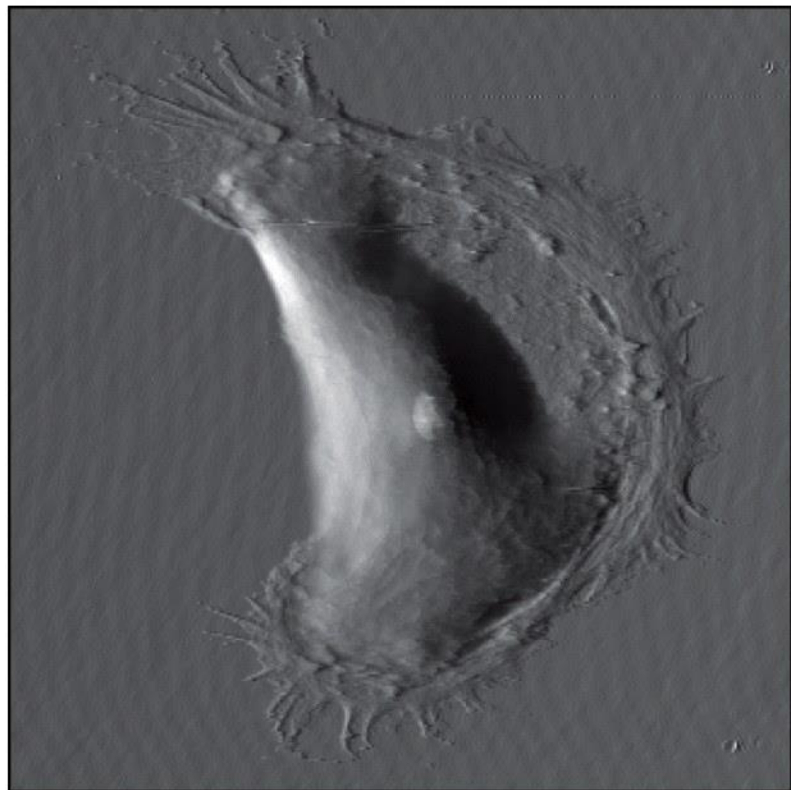
Substance	% of total dry weight	Number of molecules
Macromolecule		
Protein	55.0	2.4×10^6
RNA	20.4	
23S RNA	10.6	19,000
16S RNA	5.5	19,000
5S RNA	0.4	19,000
Transfer RNA (4S)	2.9	200,000
Messenger RNA	0.8	1,400
Phospholipid	9.1	22×10^6
Lipopolysaccharide	3.4	1.2×10^6
DNA	3.1	2
Murein	2.5	1
Glycogen	2.5	4,360
Total macromolecules	**96.1**	
Small molecules		
Metabolites, building blocks, etc.	2.9	
Inorganic ions	1.0	
Total small molecules	**3.9**	

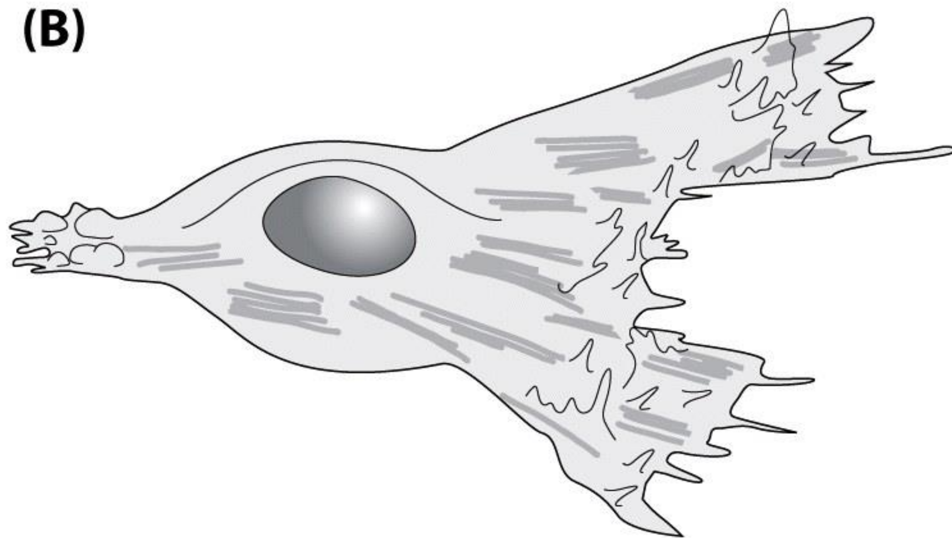
Table 2.1 Observed macromolecular census of an *E. coli* cell. (Data from F. C. Neidhardt et al., Physiology of the Bacterial Cell, Sunderland, Sinauer Associates Inc., 1990 and M. Schaechter et al., Microbe, Washington DC, ASM Press, 2006.)

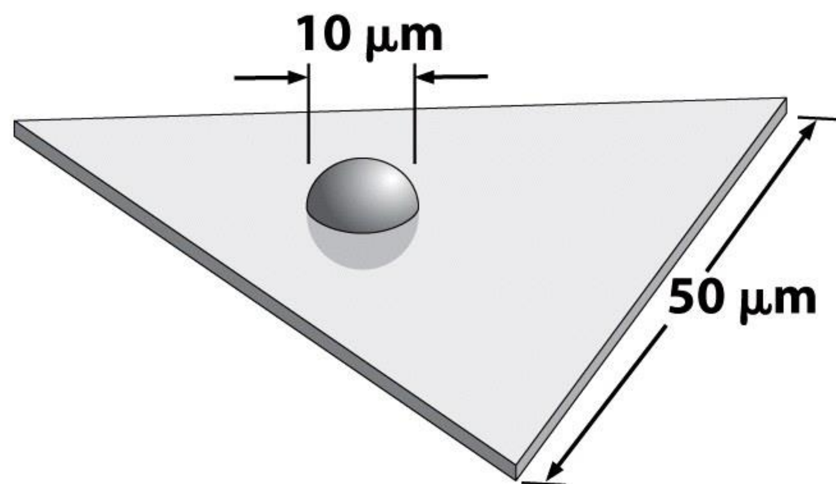
Table 2.1 Physical Biology of the Cell (© Garland Science 2009)

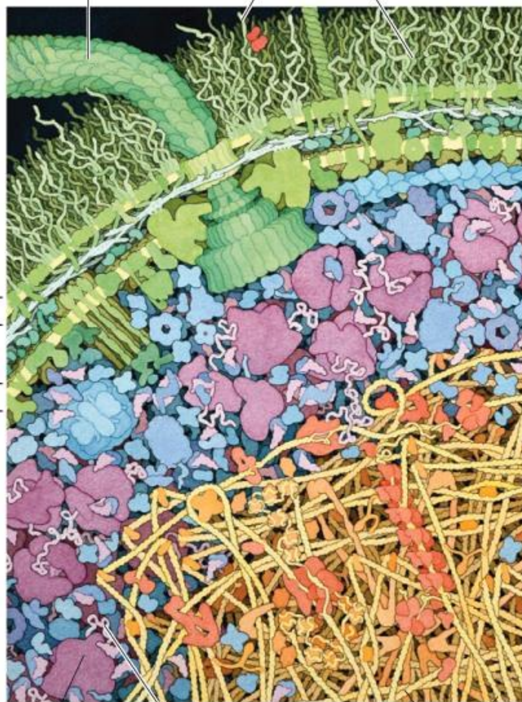
Eukaryotic Cell

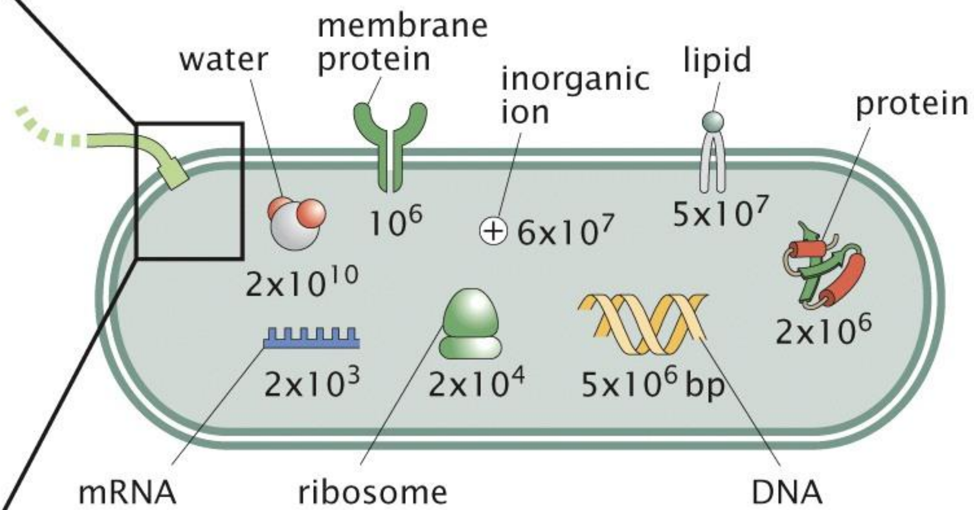



(A)
20 μm


(B)


(C)
10 μm
50 μm


flagellum
lipopolysaccharide
outer membrane
inner membrane
ribosome
mRNA
DNA


water
membrane protein
inorganic ion
lipid
protein
$2x10^{10}$
10^6
$6x10^7$
$5x10^7$
$2x10^6$
$2x10^3$
$2x10^4$
$5x10^6$ bp
mRNA
ribosome
DNA

(A)

PCR product

TAG

selectable marker

unique targeting sequences

homologous recombination

chromosome

gene 1

gene 2

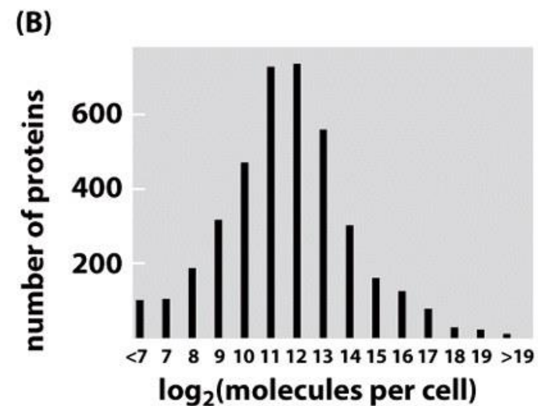
N protein 1 TAG C

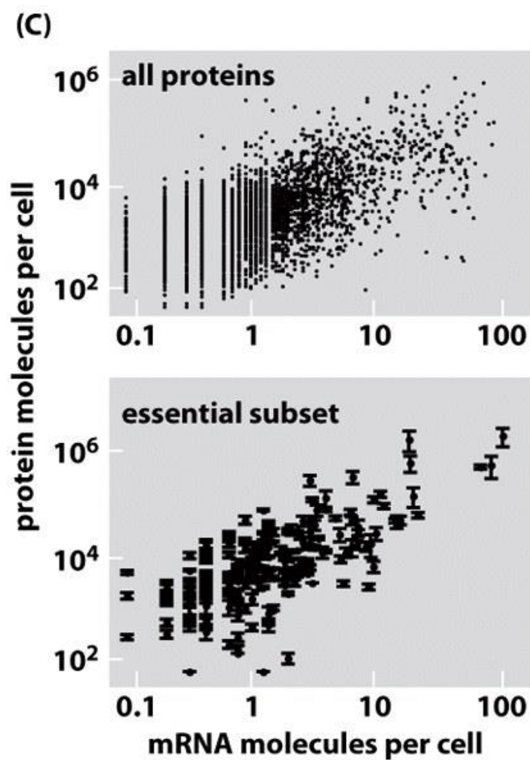
~6000 strains

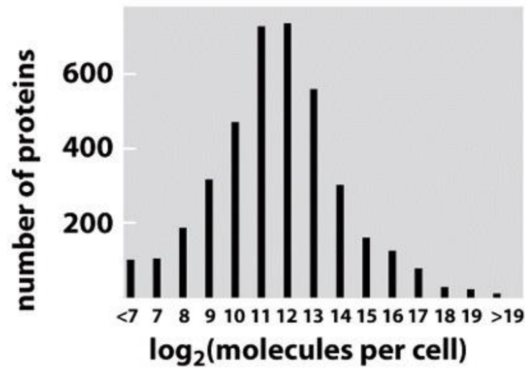
tagged proteins detected with specific antibody

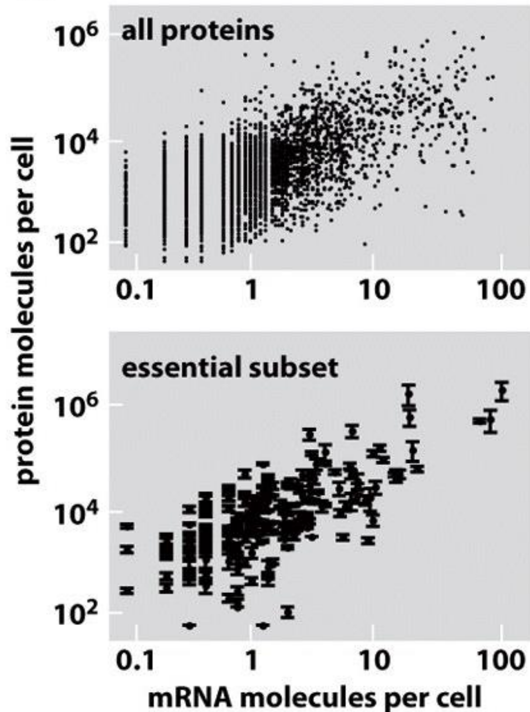
GFP measured with a fluorimeter

protein localization determined with a microscope









An important question you can ask yourself is the following how this plot changes during the cell cycle?
Can we observe protein expression in real time?
How these expression pattern change over time?

Probing protein heterogeneity in the plasma membrane using PALMand pair correlation analysis

Prabuddha Sengupta[1,4], Tijana Jovanovic-Talisman[1,3,4], Dunja Skoko[1], Malte Renz[1], Sarah L Veatch[2] & Jennifer Lippincott-Schwartz[1]

Photoactivated localization microscopy (PALM) is a powerful approach for investigating protein organization, yet tools for quantitative, spatial analysis of PALMdatasets are largely missing. Combining pair-correlation analysis with PALM (PC-PALM), we provide a method to analyze complex patterns of protein organization across the plasma membrane without determination of absolute protein numbers. The approach uses an algorithm to distinguish a single protein with multiple appearances from clusters of proteins. This enables quantification of different parameters of spatial organization, including the presence of protein clusters, their size, density and abundance in the plasma membrane. Using this method, we demonstrate distinct nanoscale organization of plasma-membrane proteins with different membrane anchoring and lipid partitioning characteristics in COS-7 cells, and show dramatic changes in glycosylphosphatidylinositol (GPI)-anchored protein arrangement under varying perturbations. PC-PALMis thus an effective tool with broad applicability for analysis of protein heterogeneity and function, adaptable to other single-molecule strategies.

show no enriched signal or specific spatial organization when visualized optically because the scale of their heterogeneity is too small to be resolved. Techniques such as electron microscopy[5,6], near-field scanning optical microscopy[7] and fluorescence resonance energy transfer[8,9], all of which can be used for nanoscale interrogation, do not offer a detailed nanoscopic description of overall plasma-membrane protein organization at high density owing to several technical obstacles. In particular, high-density labeling of proteins in electron microscopy is difficult, antibodies used in electron microscopy and near-field scanning optical microscopy may cause artifactual cross-linking (or result in multiple labeling of single proteins)[10], mechanical rip-off of the top portion of the cell to visualize plasma-membrane proteins in electron microscopy could well disrupt plasma-membrane nanoscale organization, and fluorescence resonance energy transfer cannot be used to interrogate protein organization over distances greater than ~10 nm.

A promising approach to overcome many of these limitations is single-molecule super-resolution imaging, involving single marker switching to enable light emission from only one fluoro-

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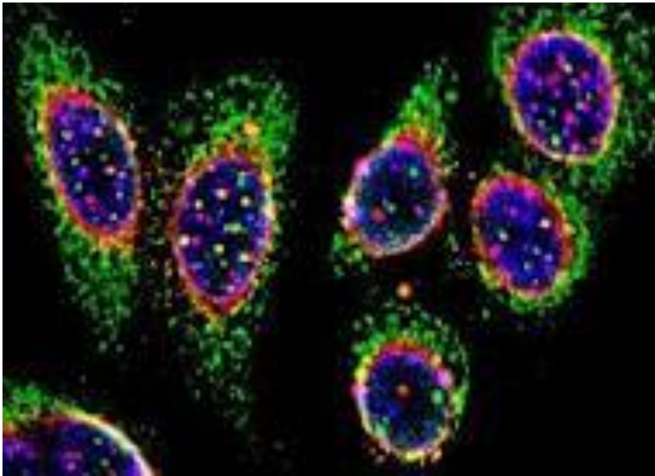
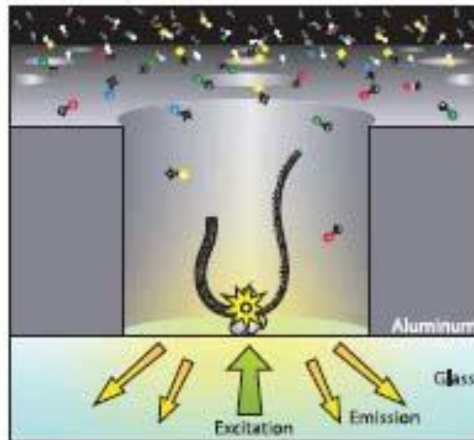
Questions :

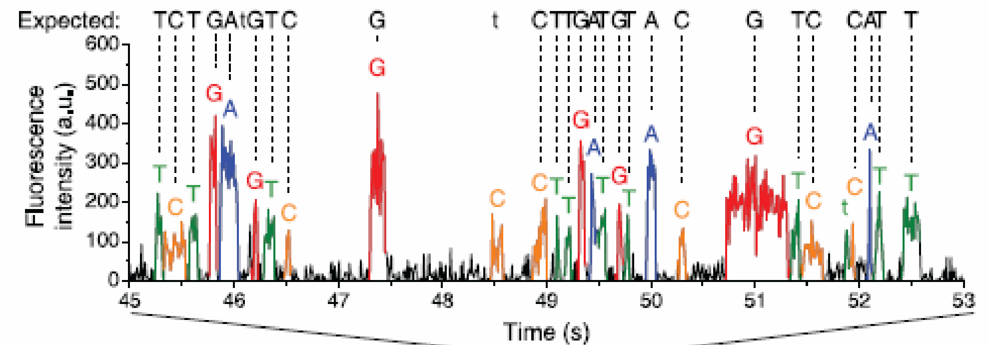
- What is the distribution of membrane protein plasma membrane?
- How can we separate cluster formation in membrane?
- What is the spatial organization in plasma membrane?

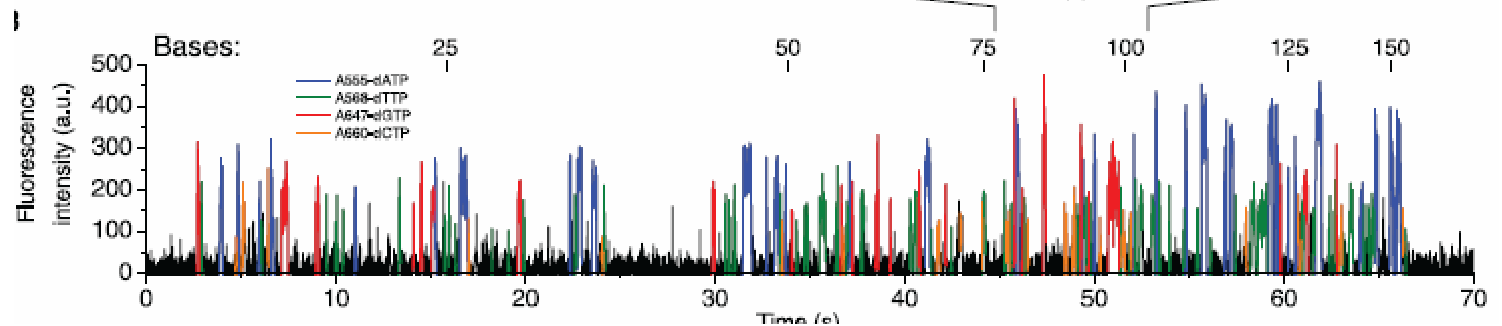


Problems to be solved:
1. Plasma membrane protein has no signal to resolve
2. Scale of heterogeneity is so small, about 10 nm. It can not be resolved regular fluorescence microscopy and confocal microscopy
3. Electron microscopy does not give any dynamic information

Single Molecule DNA Sequencing Consists of Many Poisson Processes in Series

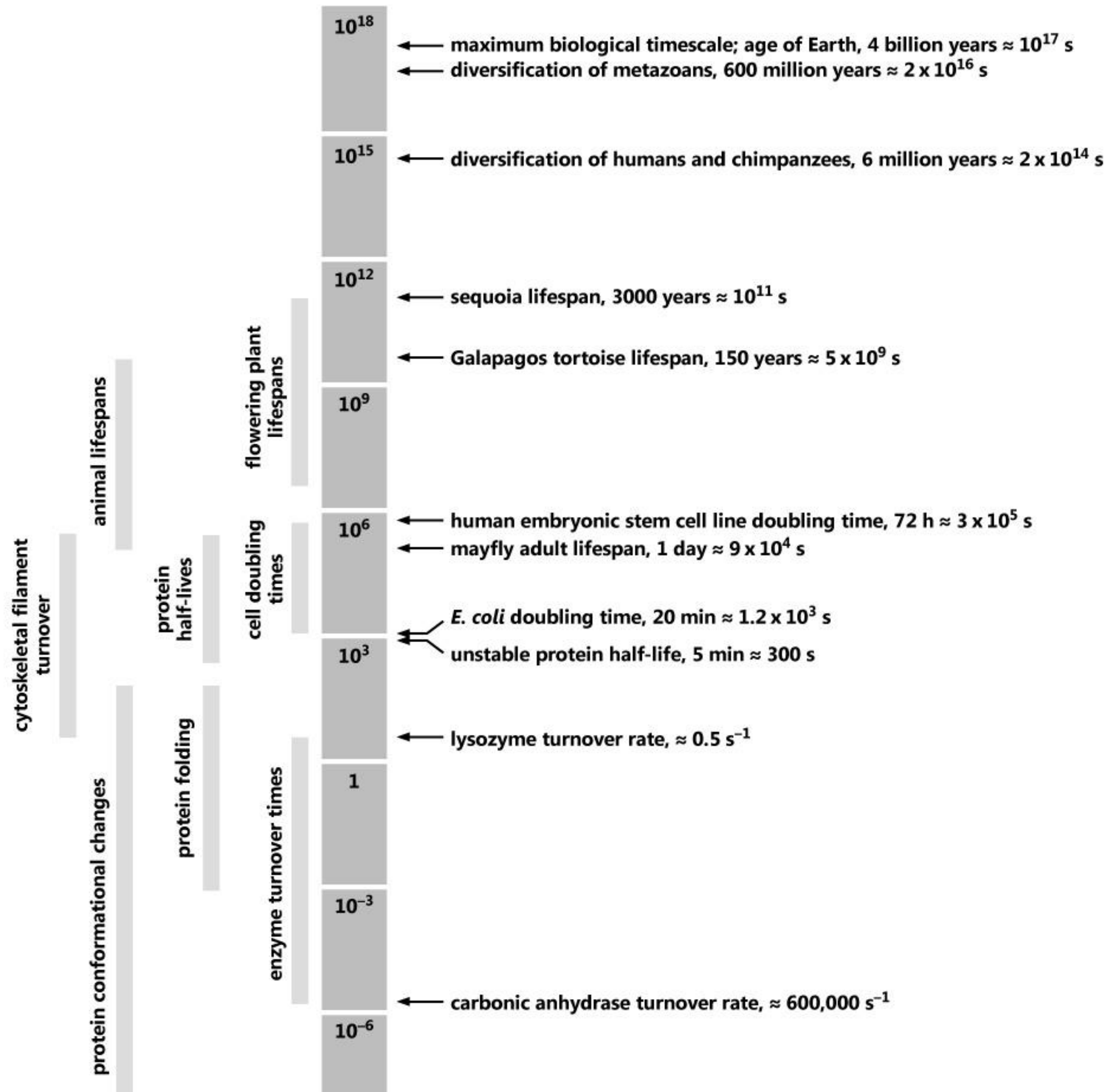






J. Korlach, S. Turner and coworkers. *Science* 323, 133 (2009)

How long does the biological processes take? – Temporal resolution in biology



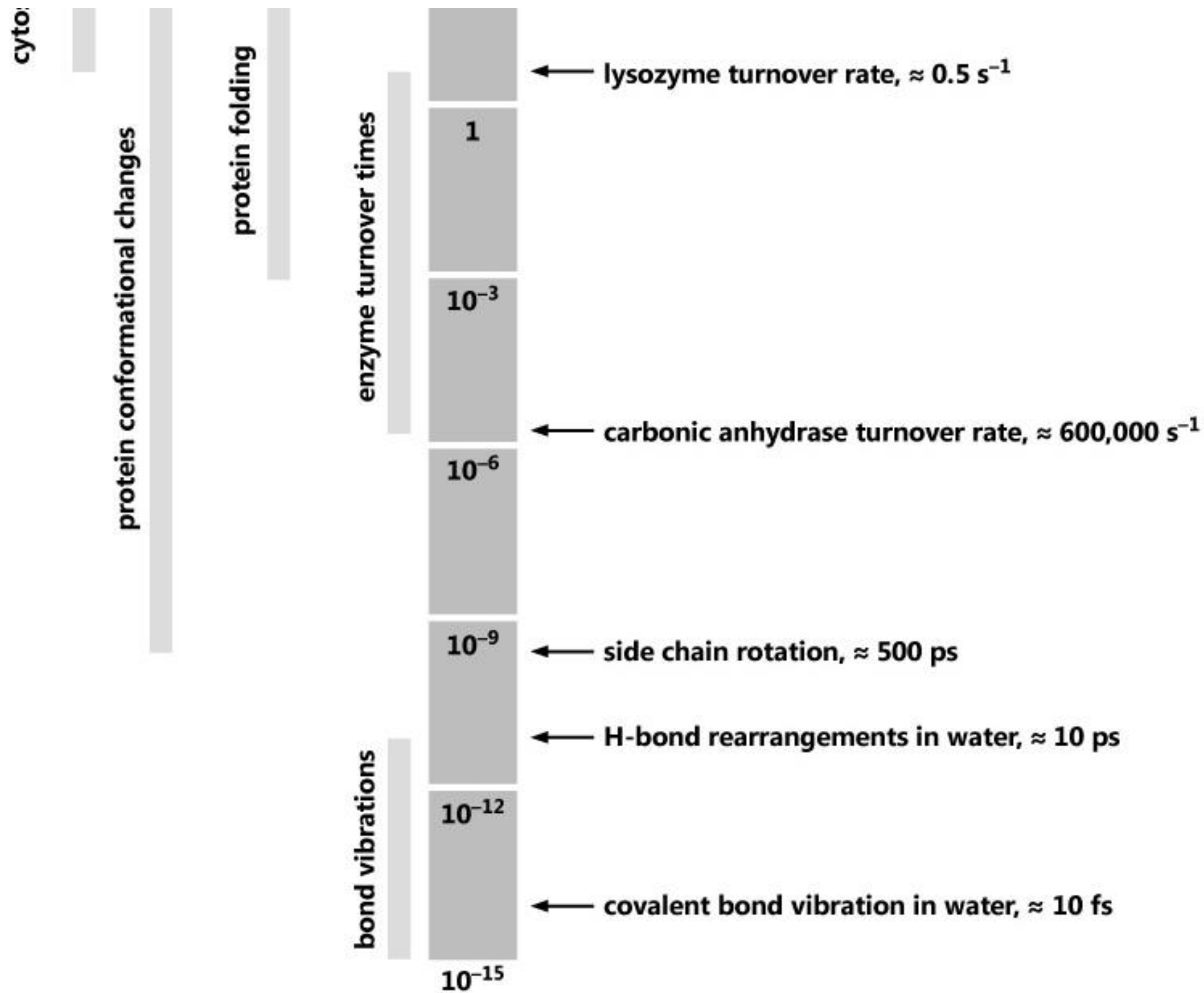


Figure 3.1 Physical Biology of the Cell (© Garland Science 2009)

Stokes-Einstein Equation

- $D = k_B T / 6\pi\gamma R = 100\ um^2/s$, diffusion constant of a single protein

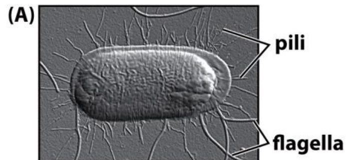
Thermal energy at room temperature

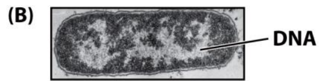
- At 25 C, $k_B T = 4.11 \times 10^{-21}$ J or Nm
- γ = viscosity of the medium, $10^{-3}\ Ns/m^2$
- R = radius of the protein, average = $2.5\ 10^{-9}$ m

Active transport vs. Passive transport

q is the dimension factor

$t_{diffusion} = x^2 / qD$





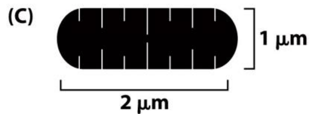
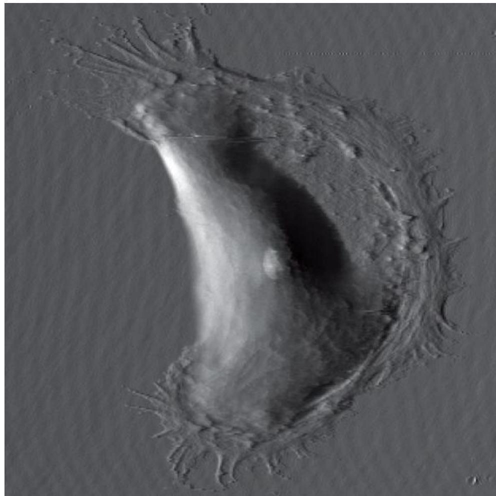


Figure 2.1 Physical Biology of the Cell (© Garland Science 2009)

Length of E.coli = 2 um

$t_{in\ e.coli} = 4\ um^2 / 2x100um^2/s = 20\ ms$, fast enough to regulate events in bacteria.

Vs.

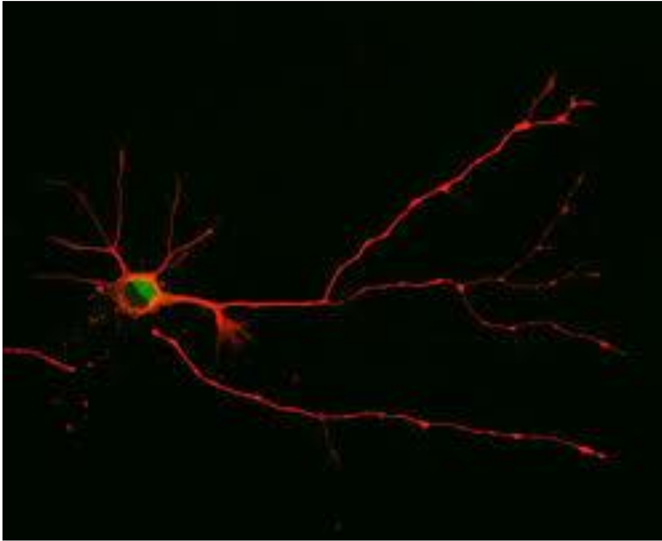


Length of cell = x = 40 um

$t_{in\ e.coli} = 1600\ um^2 / 2x100um^2/s = 8\ s$

Very slow process to transport proteins and organelles in the cell

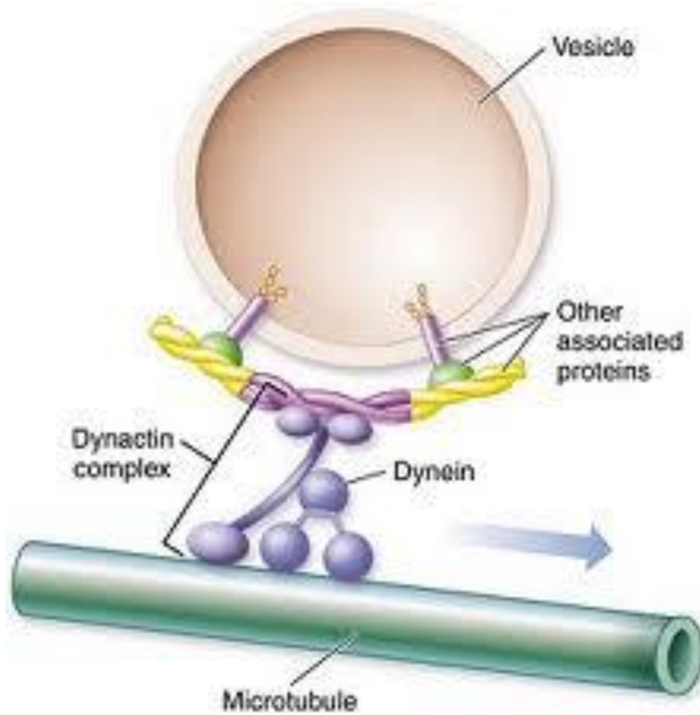
Axon

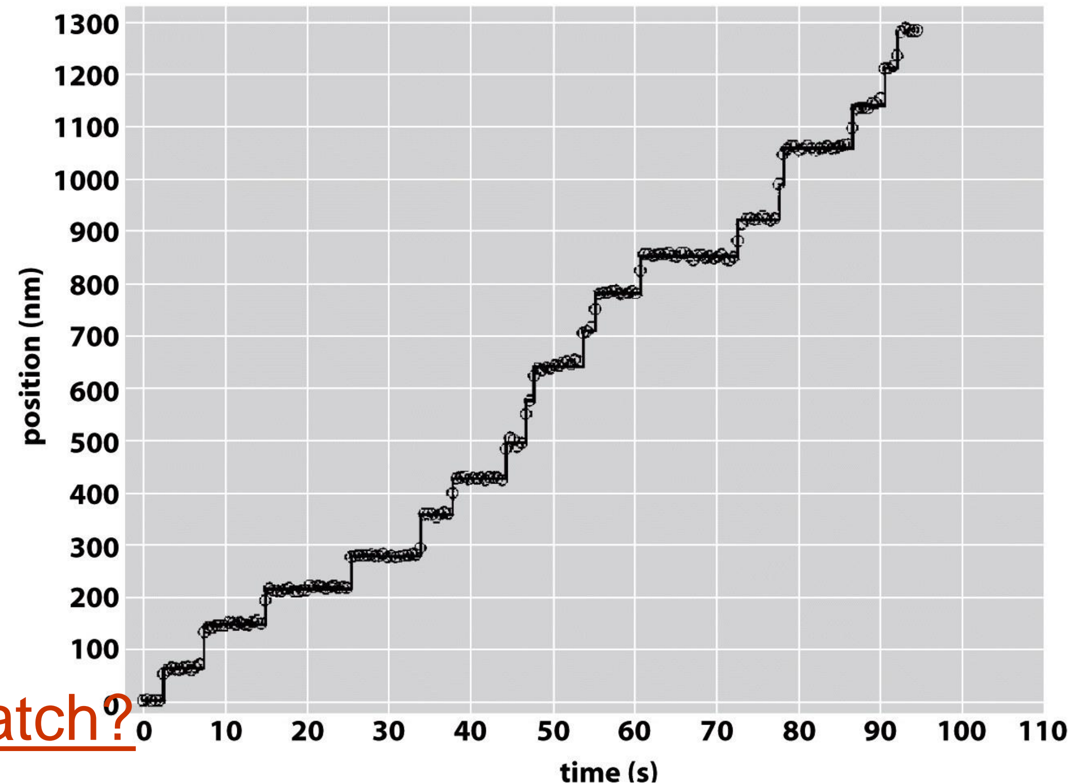


Suppose length of axon 200 um

$t_{in\ e.coli}$ = 40000 um^2 / 2x100um^2/s = 200 s
Very very slow process to transport proteins and organelles in the cell

Active transport system to solve the problem!!
Kinesin moves along the microtubule.

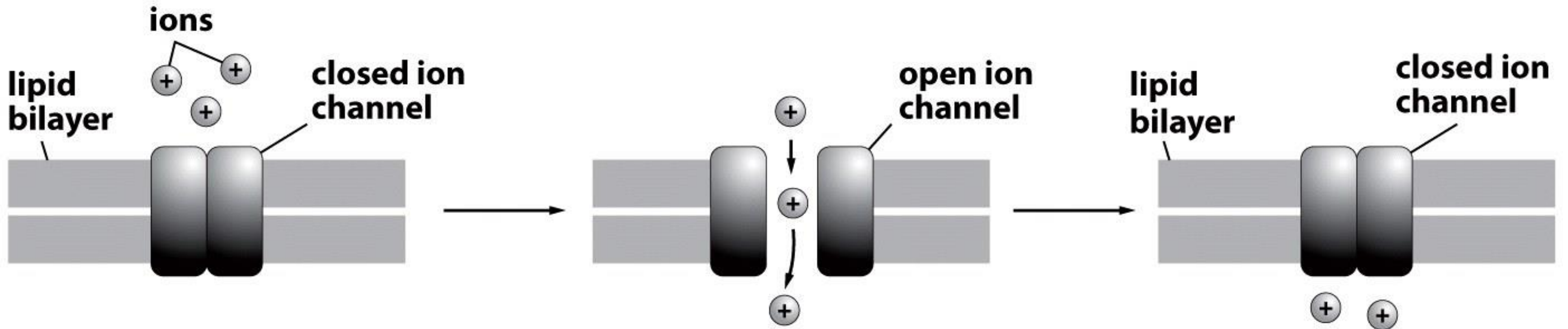




http://www.youtube.com/watch?v=pt53JOXP-GE

gating of ion channels

Millisecods



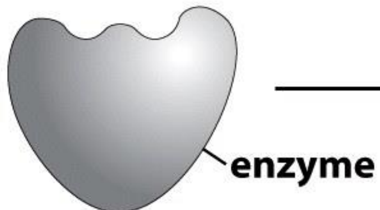
enzyme catalysis

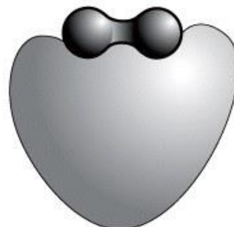
0.0 x 10^{-6} seconds

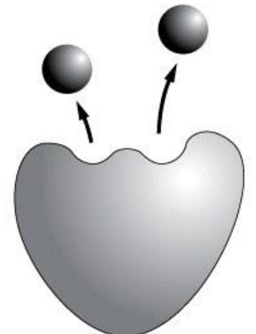
0.1 x 10^{-6}

1.0 x 10^{-6}

substrate







bacterial cell division

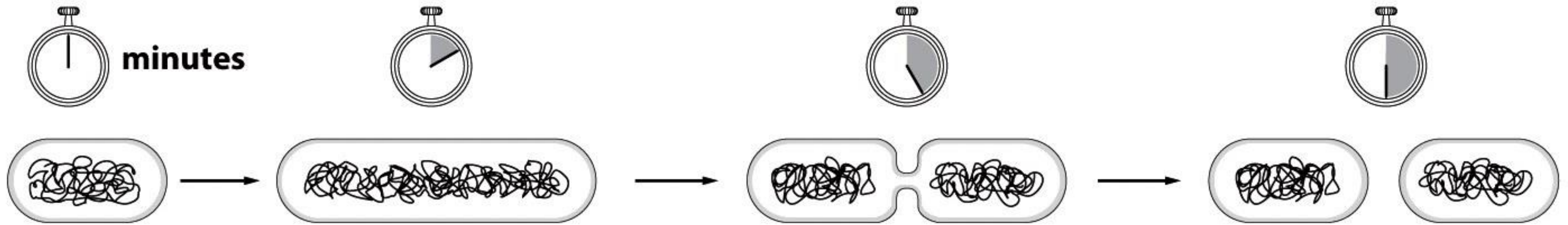


Figure 3.2c Physical Biology of the Cell (© Garland Science 2009)

cell movements

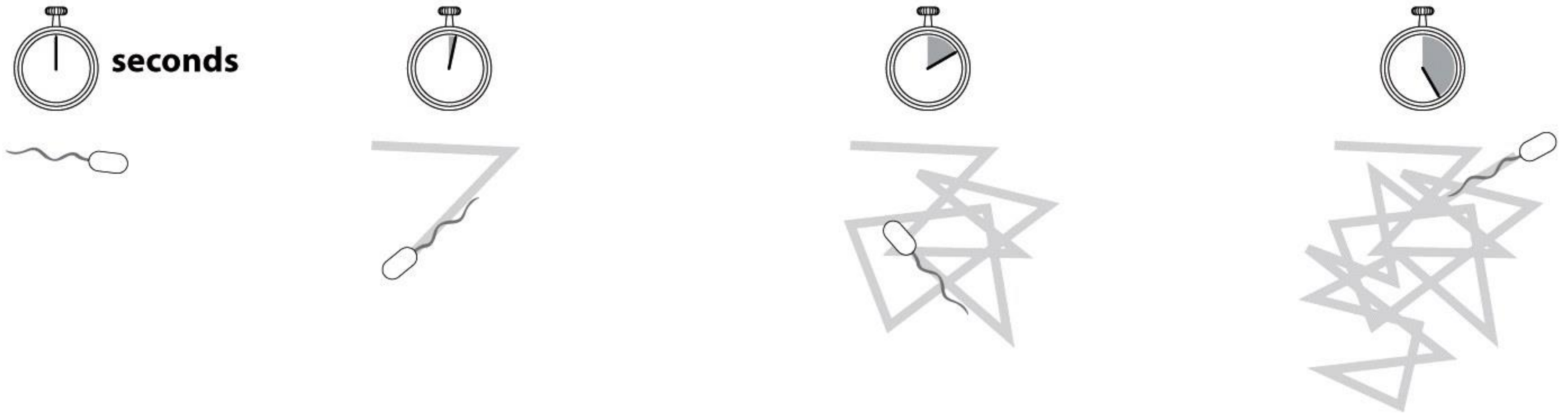


Figure 3.2d Physical Biology of the Cell (© Garland Science 2009)

protein synthesis

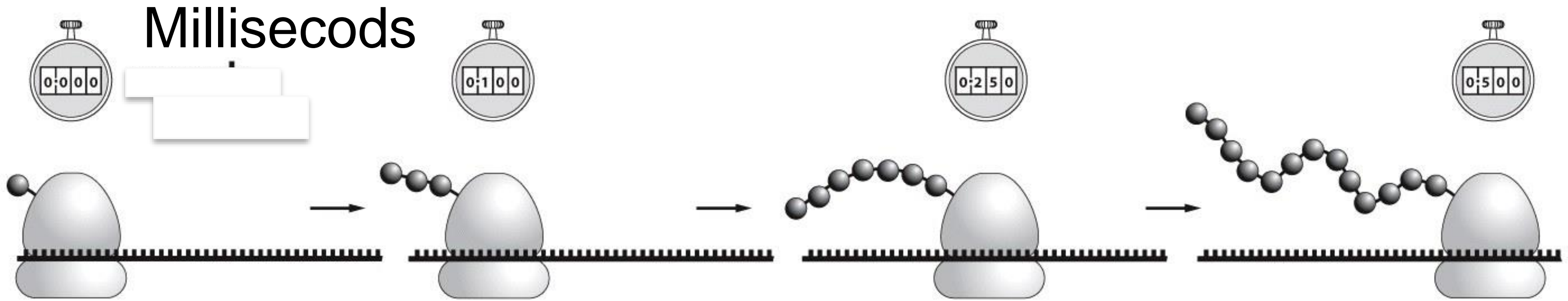


Figure 3.2e Physical Biology of the Cell (© Garland Science 2009)

transcription

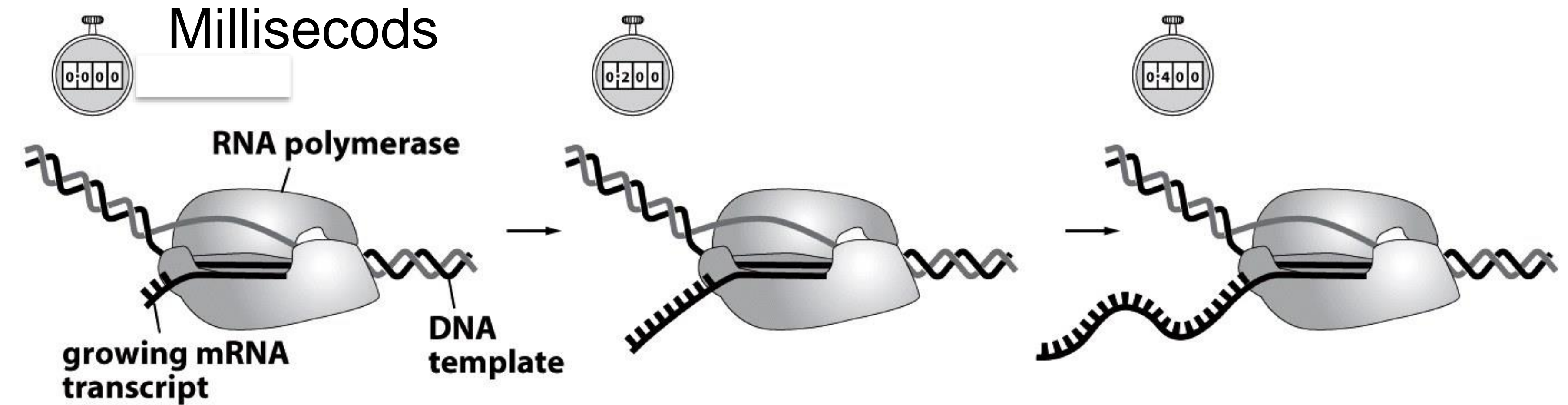
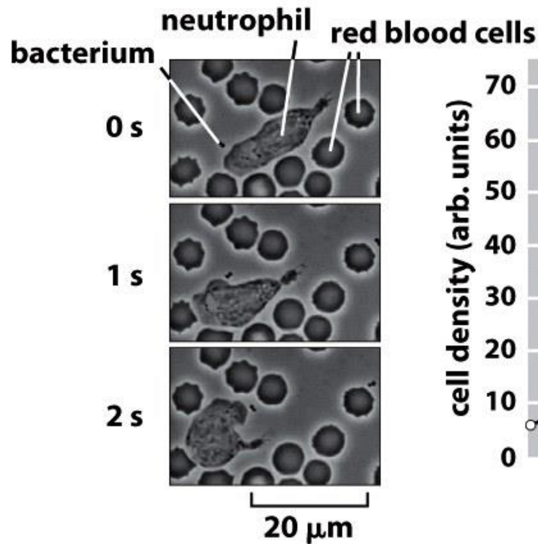
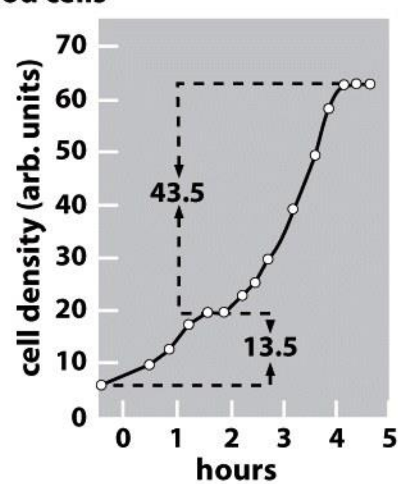
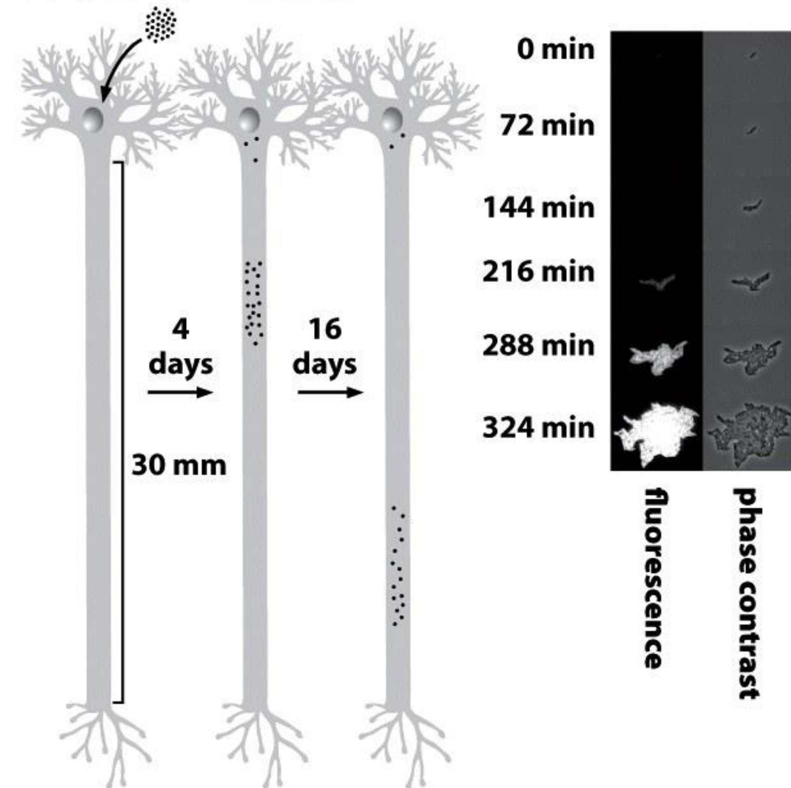


Figure 3.2f Physical Biology of the Cell (© Garland Science 2009)

METHOD	direct observation	fixed time points	pulse–chase	product accumulation
TYPICAL TIME SCALES	milliseconds to hours	microseconds to years	minutes to days	minutes to days
TYPES OF PROCESSES	individual transformations	population changes	continuous (e.g., metabolism, transport)	biosynthetic or enzymatic
EXAMPLE	cell crawling	bacterial growth curve	axonal transport	GFP expression

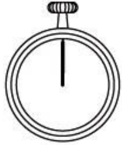


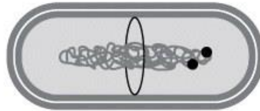


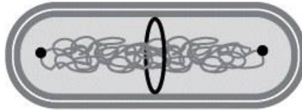


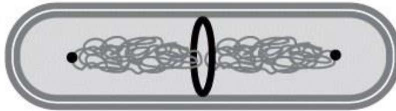
E.Coli cells divide in 40-50 minutes.

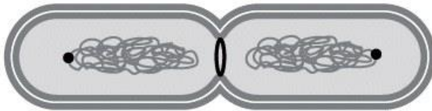
minutes

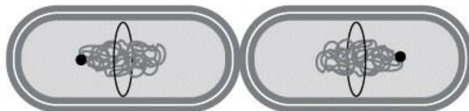


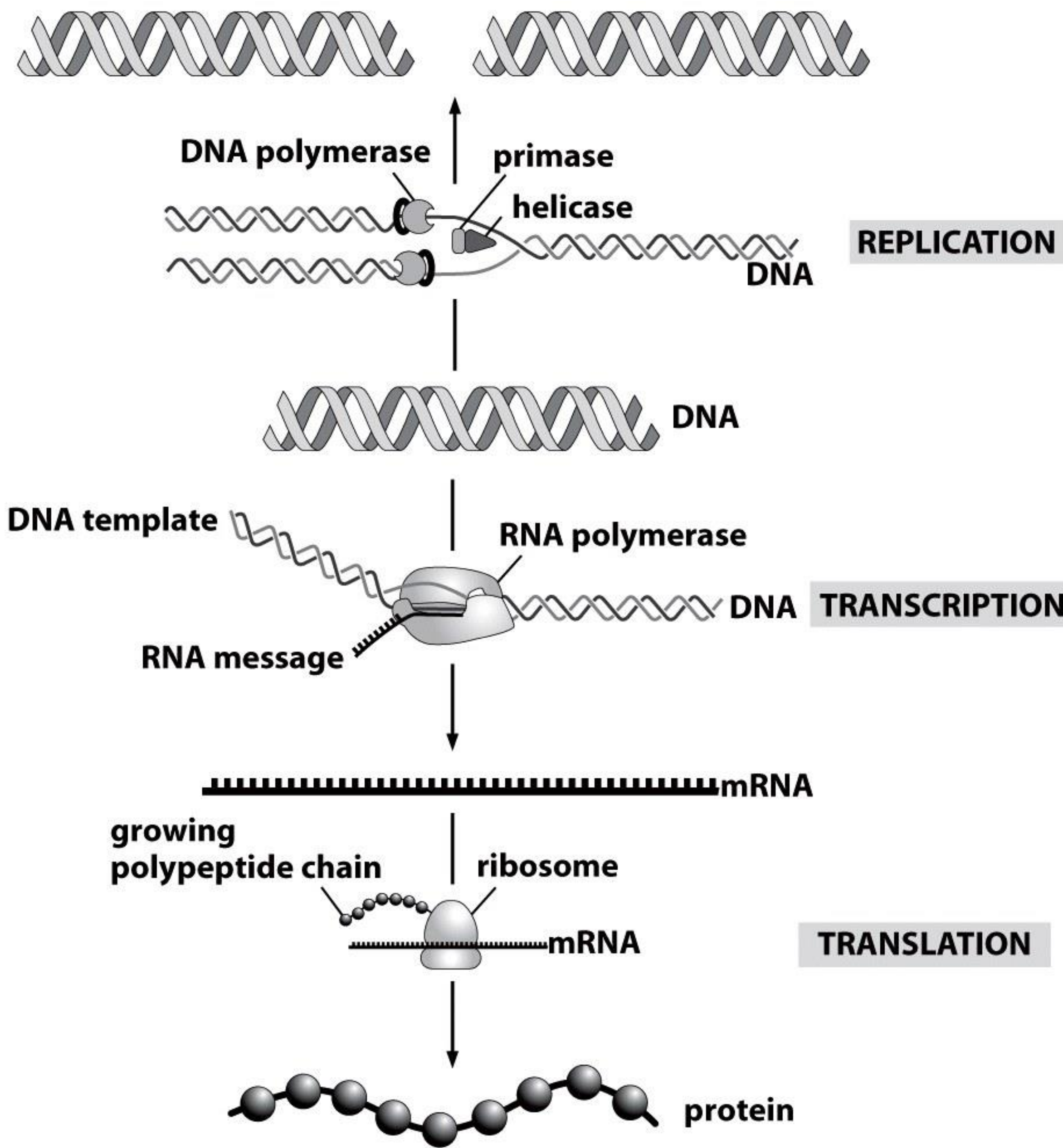











DNA polymerase
primase
helicase
REPLICATION
DNA
DNA
DNA template
RNA polymerase
DNA
TRANSCRIPTION
RNA message
mRNA
growing
polypeptide chain
ribosome
mRNA
TRANSLATION
protein

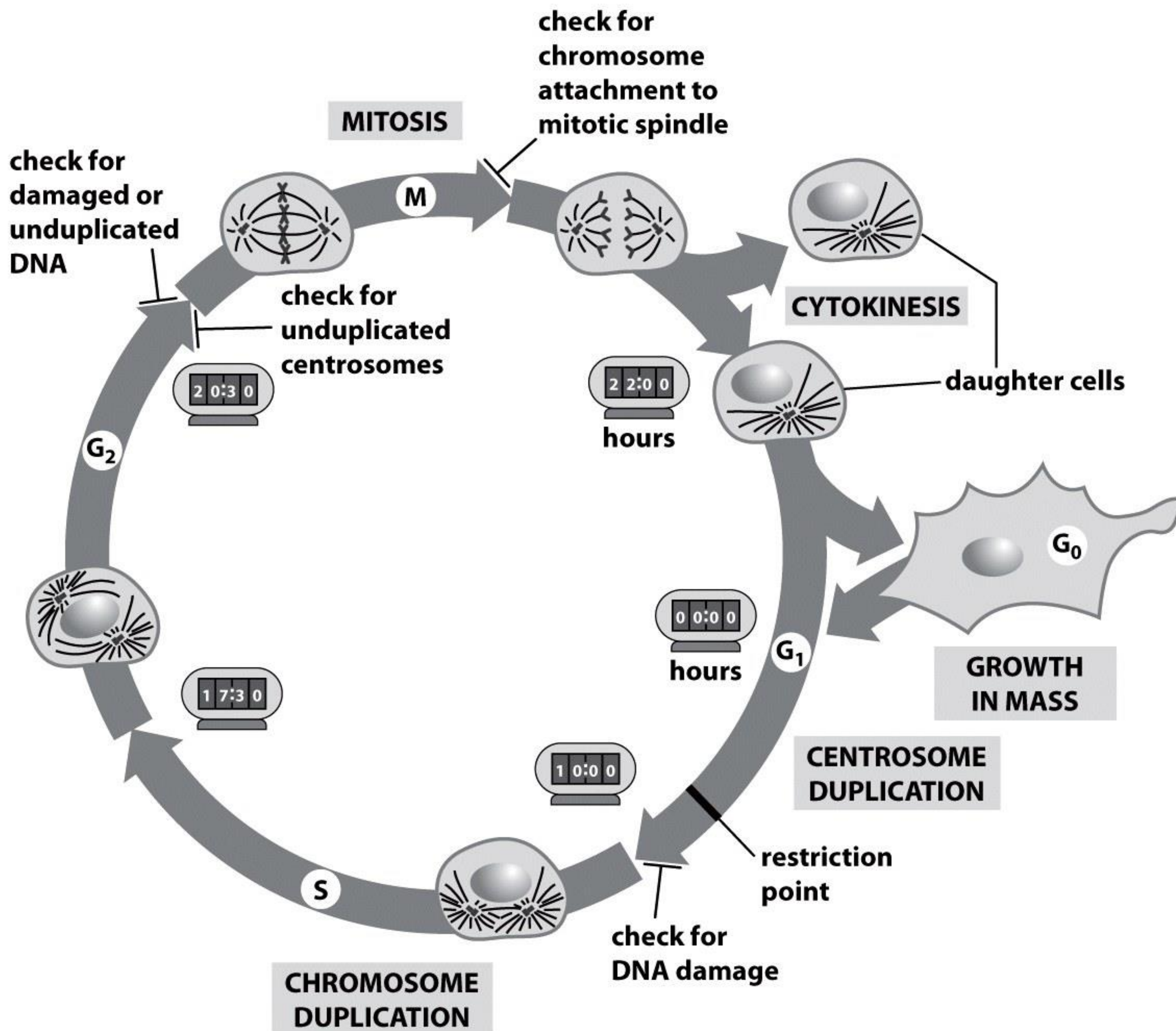


Figure 3.15a Physical Biology of the Cell (© Garland Science 2009)

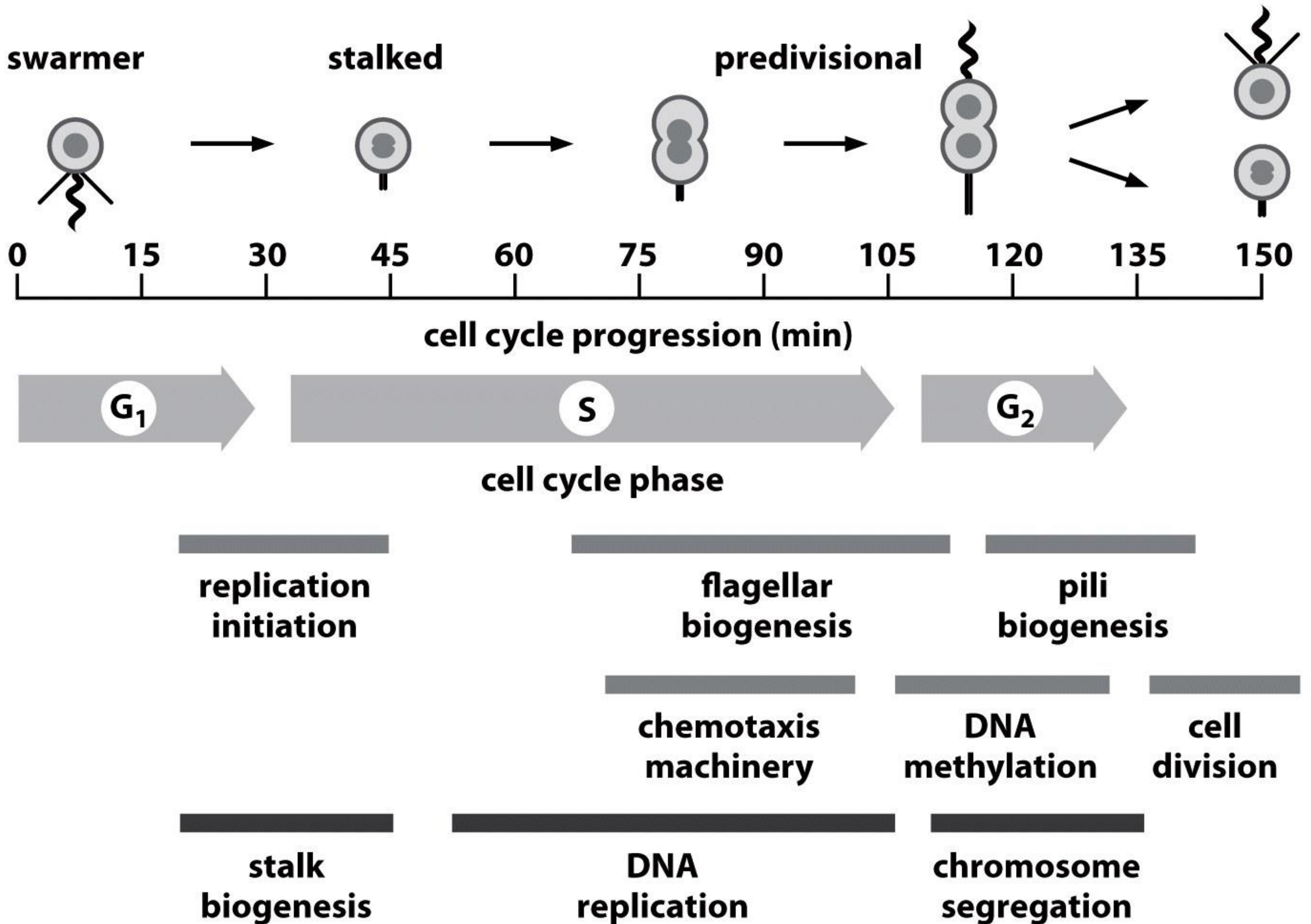


Figure 3.17a Physical Biology of the Cell (© Garland Science 2009)

Tracking the virus entry into cells in real time

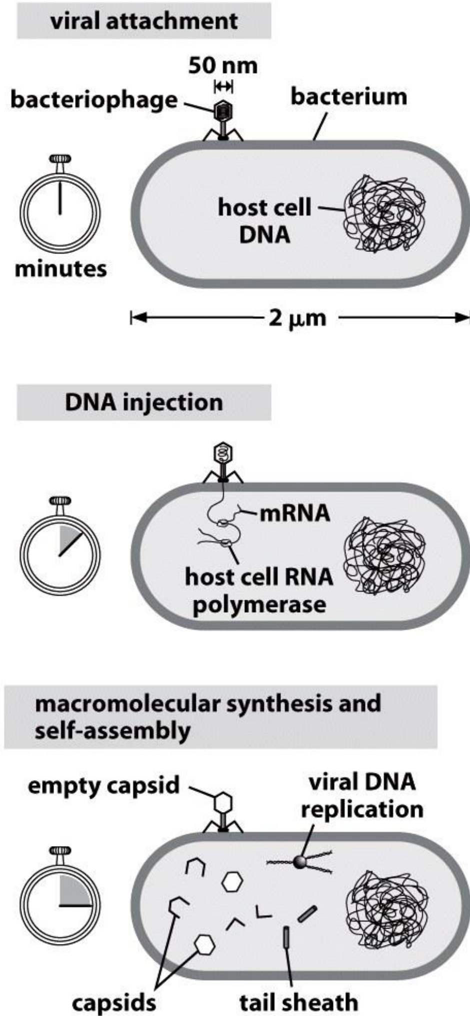
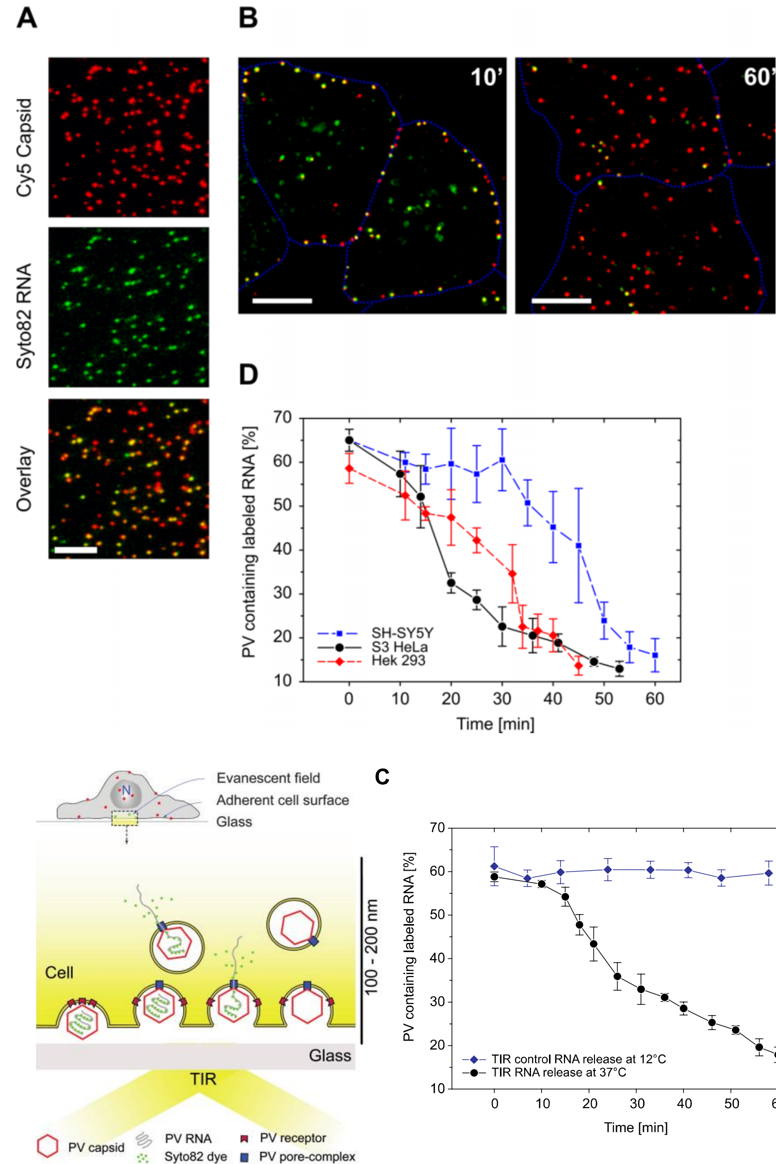


Figure 3.20 Physical Biology of the Cell (© Garland Science 2009



B. Brandenburg, L. Y. Lee, M. Lakadamyali, M. J. Rust, X. Zhuang, J. M. Hogle, "Imaging poliovirus entry in live cells", ***PLoS Biol.* 5**, e183, 1543-1555 (2007)