

Volta Flame – tonight 7pm BBB/Baxter Pond



Prof. Jared Leadbetter



Alessandro Volta



Microbial Diversity Course, Woods Hole

Draw out a methanogen write $\text{CO}_2 + 4 \text{H}_2 \rightarrow \text{CH}_4 + 2\text{H}_2\text{O}$

25% of you have methanogens in your gut (dragons!)

Outline for today

Part 1

- Introduction to different cell types
- Cell composition
- Cell dynamics/ transport
- Compartmentalization

iClicker Q1, short break

Part 2

- The eukaryotic cell
 - Features and consequences
 - Origin
- Multicellularity
 - Where and when it occurs (all domains)
 - Molecular basis

iClicker Q2

Introduction to different cell types

Bacteria, Archaea, Eucaryotes

- *Draw out cells (size) 1 vs. ~ 10 μm*
- *Compact vs. very compartmentalized*
- *No Prokaryotes!! (Will cover next week!)*



vs. Viruses, Prions, Viroids

Structural/functional v. chemical similarity

→ Enables antibiotics to target specific pathogens!

Basic principles: what does a cell need?

Functional goal

- Reproduction (make copies)
- Ability to move (get good stuff, avoid bad)
- Transportation network (get things in/out)
- Ability to harvest energy
- Ability to defend itself
- Emergency preparedness (repair damage)
- Separate the inside from outside

Components

nucleic acids

flagella, pili (proteins)

protein channels

small molecules, membranes (battery)

small molecules

proteins

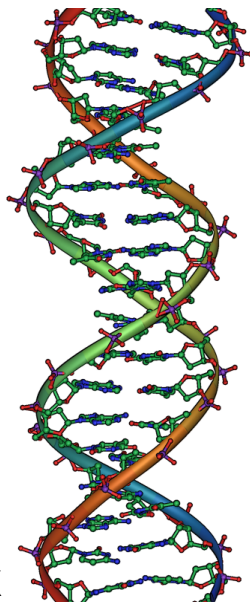
lipids, polysaccharides

Building blocks – **Nucleic acids**

Function: DNA (information storage), RNA (information transfer, catalysis, synthesis, regulation)

Polymers of nucleotides joined by sugar and phosphate backbones

- * DNA = deoxyribonucleic acid (A pairs with T, C pairs with G)
- * RNA = ribonucleic acid (AUGC) (A pairs with U, C pairs with G)



DNA double helix



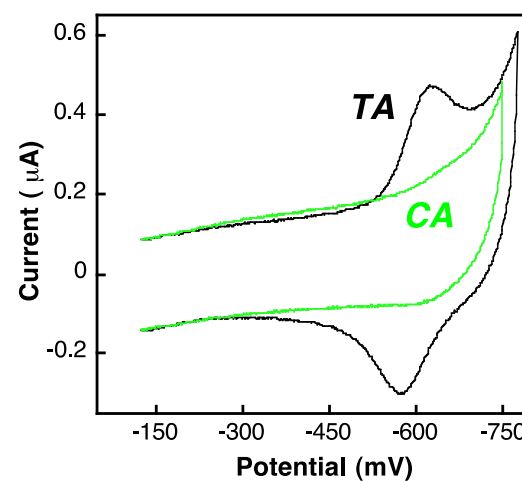
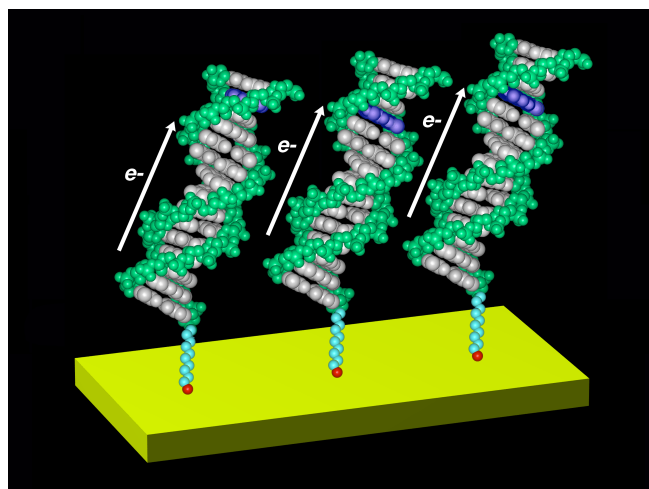
tRNA

Building blocks – **Nucleic acids**

J. Barton CCE Chair



DNA can transfer charge



* Potential *in vivo* relevance in DNA lesion detection by DNA repair proteins

Kelley, S.O.; Jackson, N.M.; Hill, M.G.; Barton, J.K. *ACI* **38**, 941 (1999).

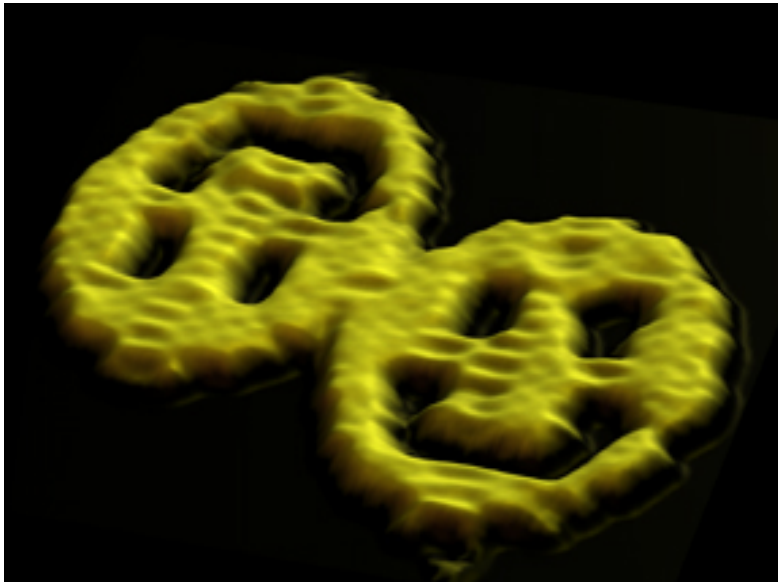
Building blocks – **Nucleic acids**

- Energetic significance of base pairing facilitates programming
(*physical modeling, computational simulation, dynamic function*)



P. Rothemund
(BE, CS)

Example: DNA “Origami” (i.e. Molecular Programming)



Giant DNA complexes imaged with AFM

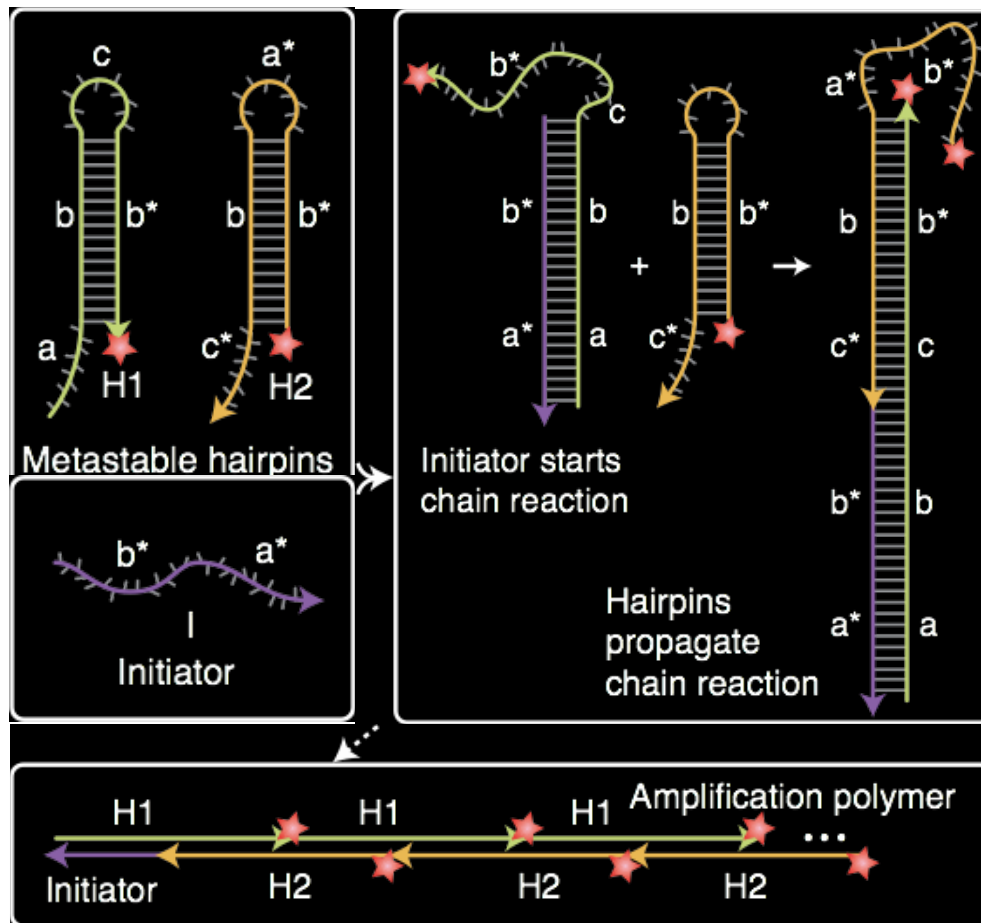
100 nm across each smiley face! Each is comprised of about 14,000 DNA bases. 7000 of these DNA bases belong to a long single strand, a DNA molecule that just happens to be the genome of the virus M13. The other 7000 of these bases belong to about 250 shorter strands, each about 30 bases long. These short strands fold the long strand into the smiley face shape.

Rothemund, Folding DNA to create nanoscale shapes and patterns, *Nature* (2006)

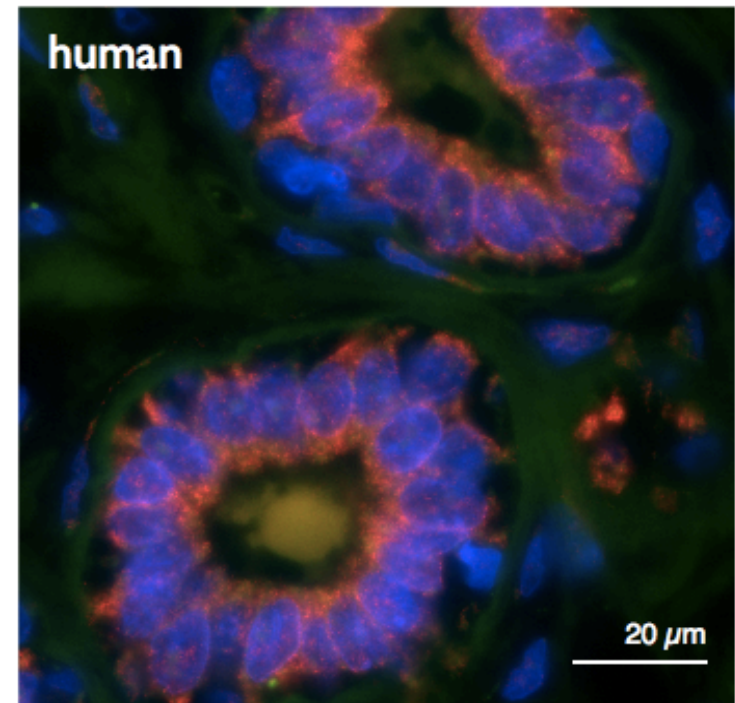
See TED talk posted online!

Building blocks – **Nucleic acids**

Example: Detection of specific RNAs



N. Pierce – BE, ACM



Dirks and Pierce, *Proc Natl Acad Sci USA*, 2004

Building blocks – **Proteins**

Function: make things, move things around, provide structure, regulate, protect and repair

20 canonical amino acids (aa):

Depending on side chain,

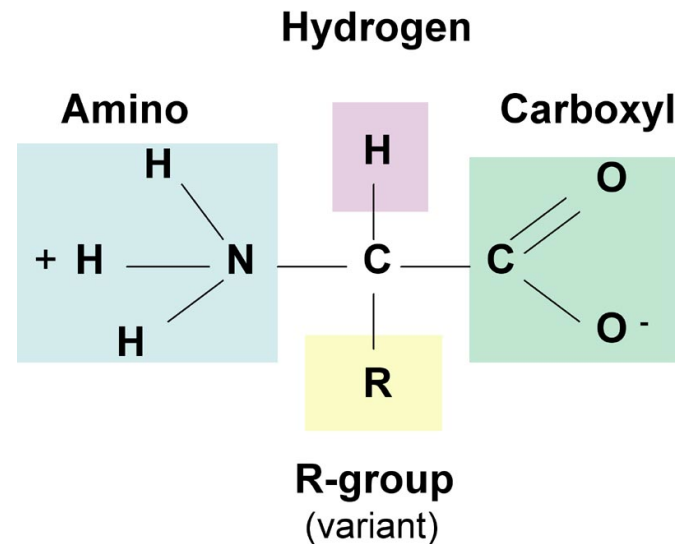
At physiological pH:

Positively or negatively charged

Uncharged

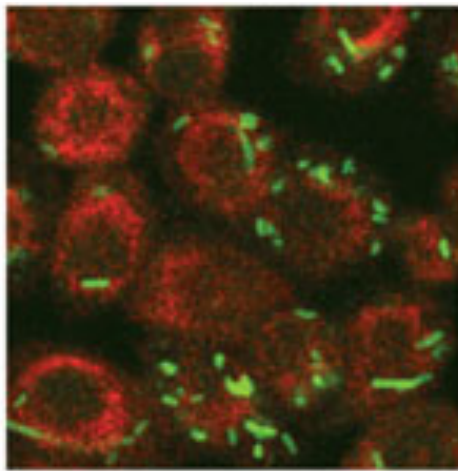
Special cases

Hydrophobic



Can we engineer new properties?

D. Tirrell (CCE)



Give example of methionine and azidonorleucine, fluorophore

JT Ngo *et al* (2009) Cell selective metabolic labeling of proteins, *Nat Chem Bio*

Building blocks – **Proteins**

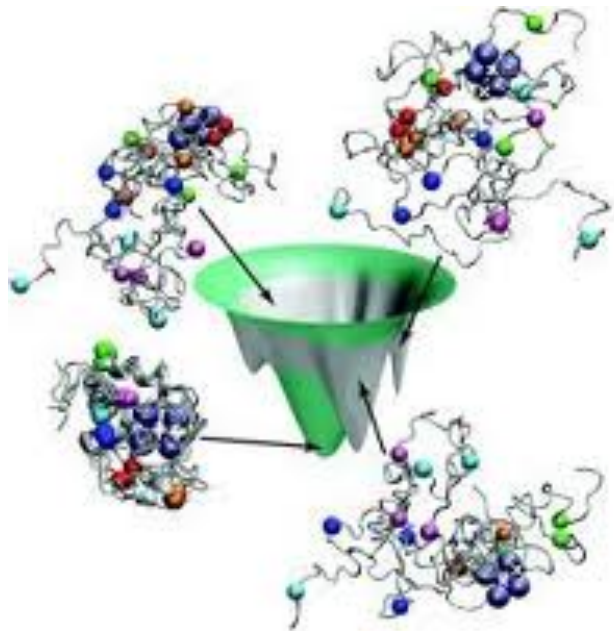
“Levinthal’s Paradox”:

Consider a small protein (polypeptide) comprised of 100 aa
Assume each aa can take on 2 different conformations (2^{100})



H. Gray (CCE)

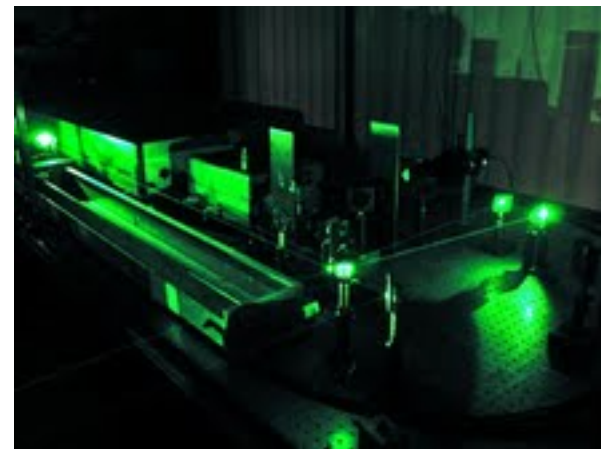
- *Time required to sample all possible conformations is roughly:*
- 10^{30} conformations $\times 10^{-12}$ s/conformation = 10^{18} s $\sim 10^{10}$ years
- *Age of the universe $\sim 1.2 \times 10^{10}$ years*



Pascher et al. *Science* **1996**, 271: 1558

Levinthal, *J. Chim. Phys.* **1968**, 65: 44

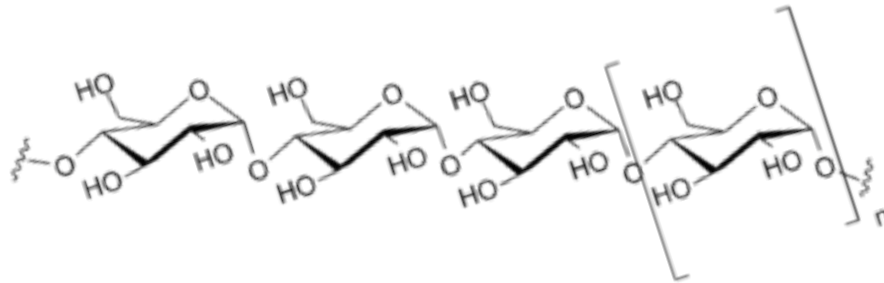
Fluorescence energy transfer
Redox-triggered protein folding!
Monitor change in Donor emission
Following 1 ps laser pulse excitation
Snapshots over 10's ps to 50 ns
Allows to extract Donor-Acceptor
Distance distributions



532 Nd:YAG laser; excite Donor

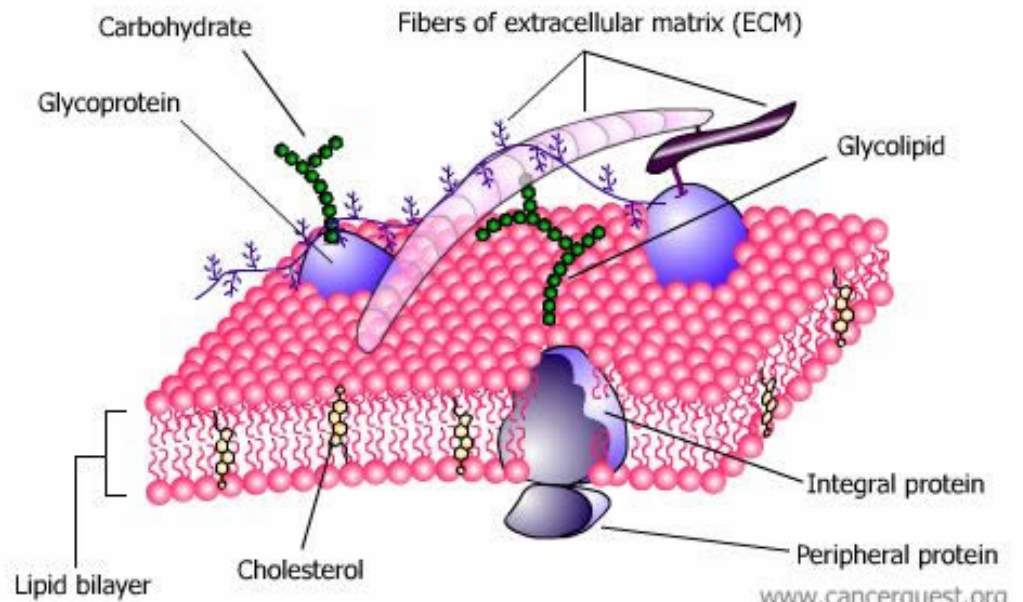
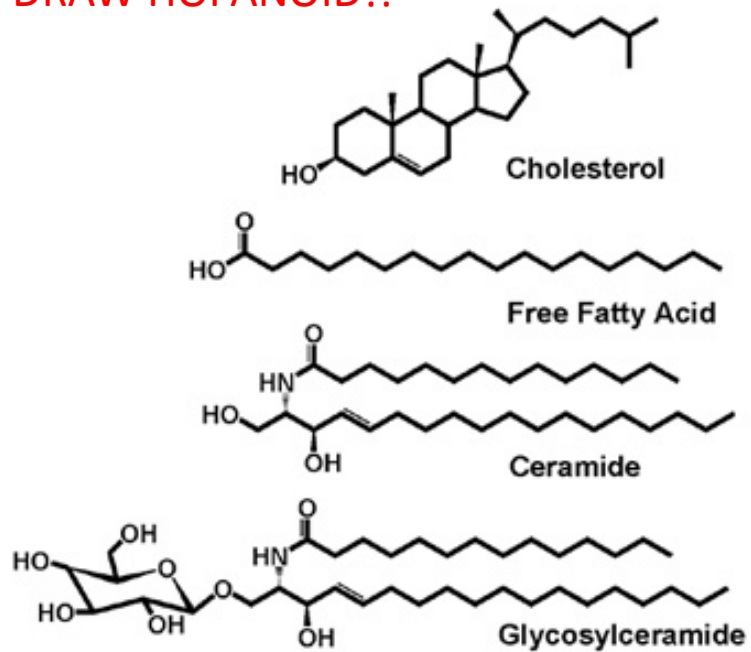
Building blocks – **Carbohydrates, Lipids**

Function: separate inside from outside

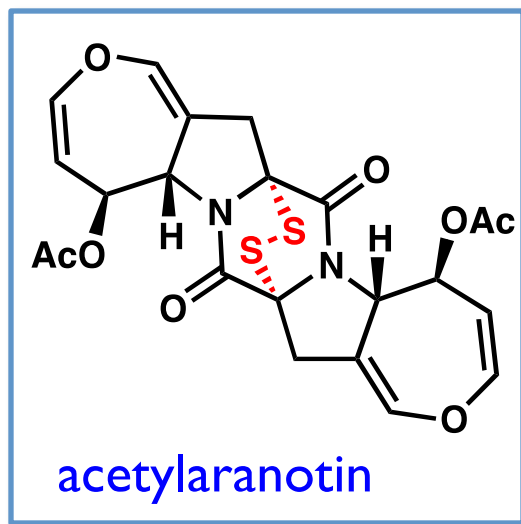


Amylose (component of starch)

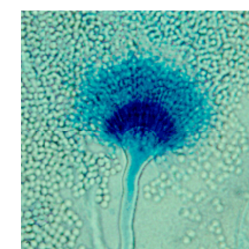
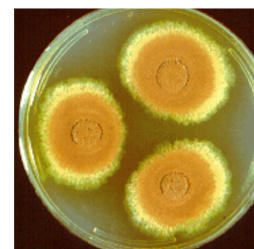
DRAW HOPANOID!!



Building blocks – **Small molecules** (function: defense)



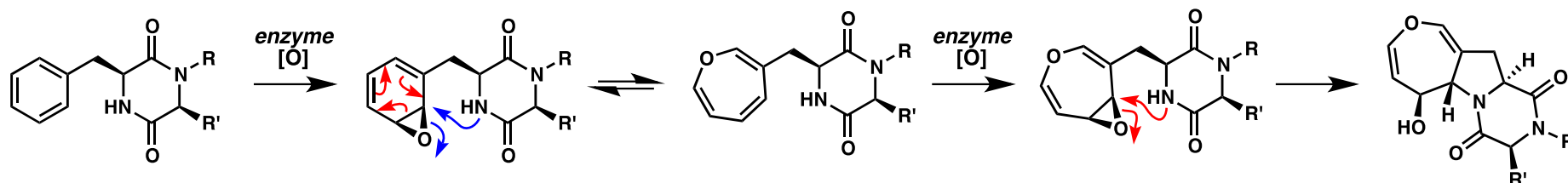
- isolated from *Arachniotus aureus* and *Aspergillus terreus* in 1968
- activity against polio, coxsackie A21, rhino- and parainfluenza viruses
- induces apoptosis in HCT116 colon cancer cells
- first total synthesis reported by Reisman and coworkers in 2012



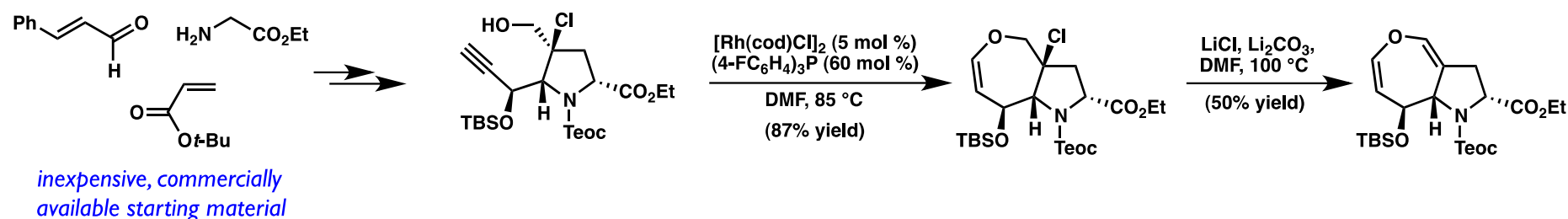
Aspergillus terreus

a producer of acetylaranotin

How the fungus does it:



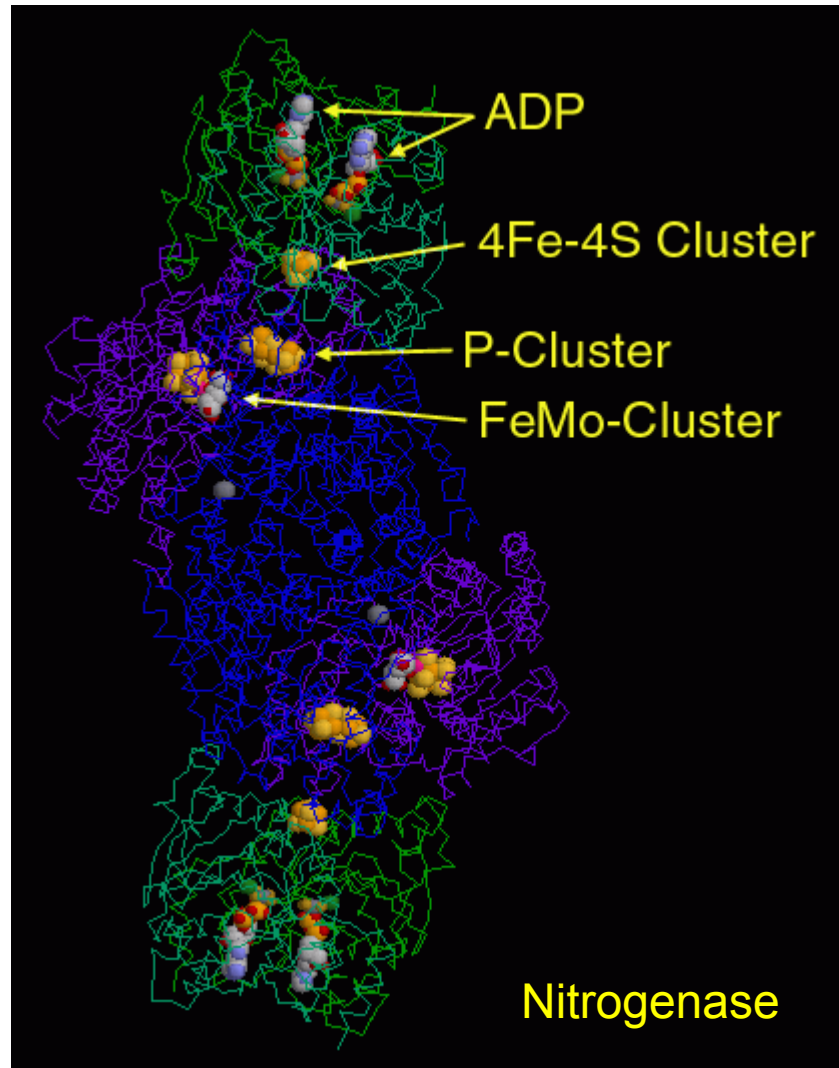
How the Reisman group does it: (requires the development of new chemical reactions!)



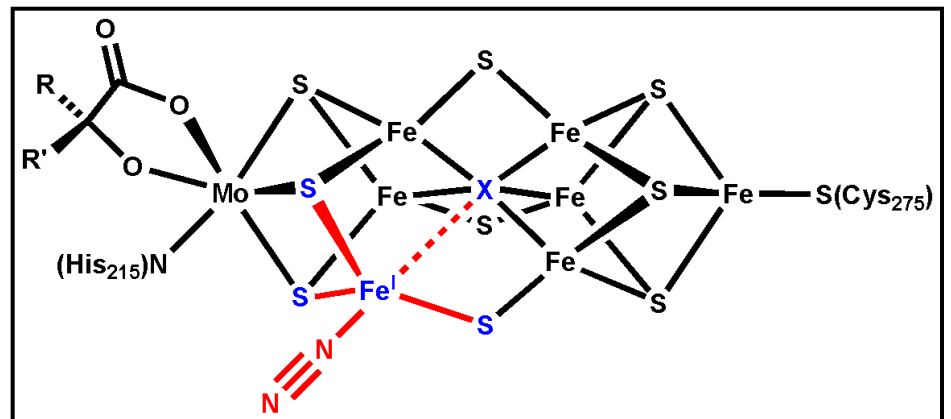
Building blocks – **metal cofactors** (function: catalysis)



J. Peters (CCE)

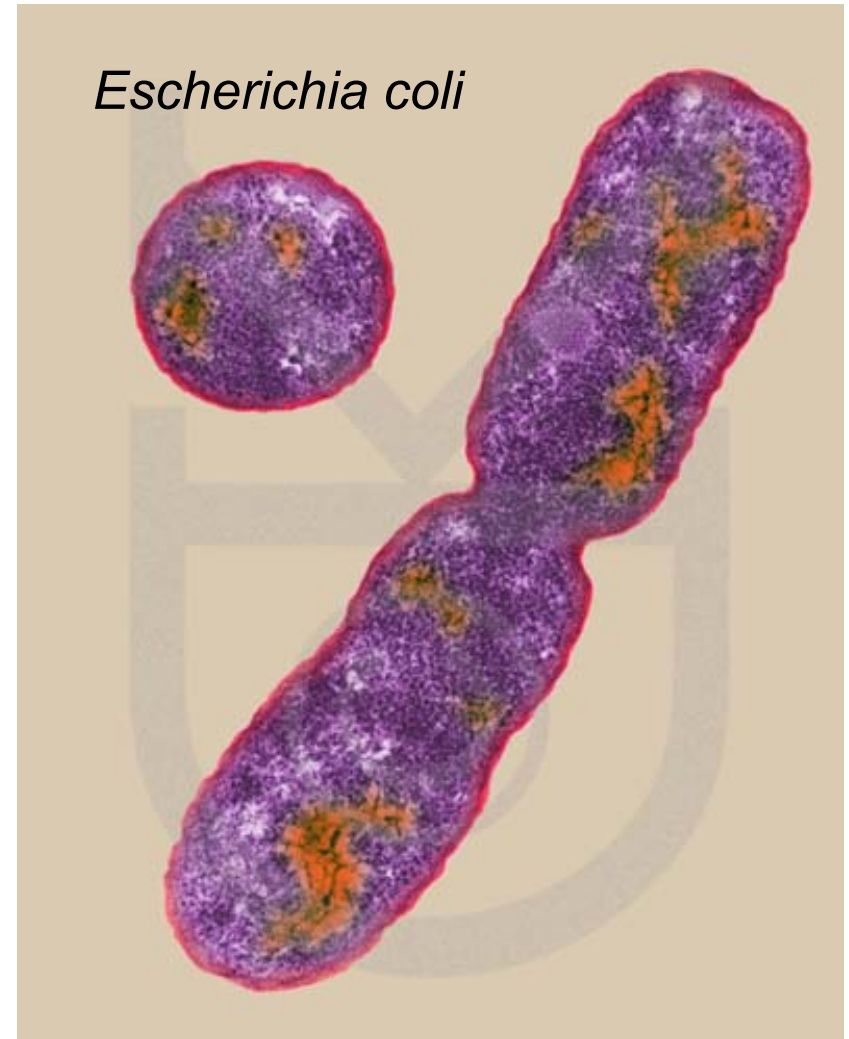


The FeMo cofactor

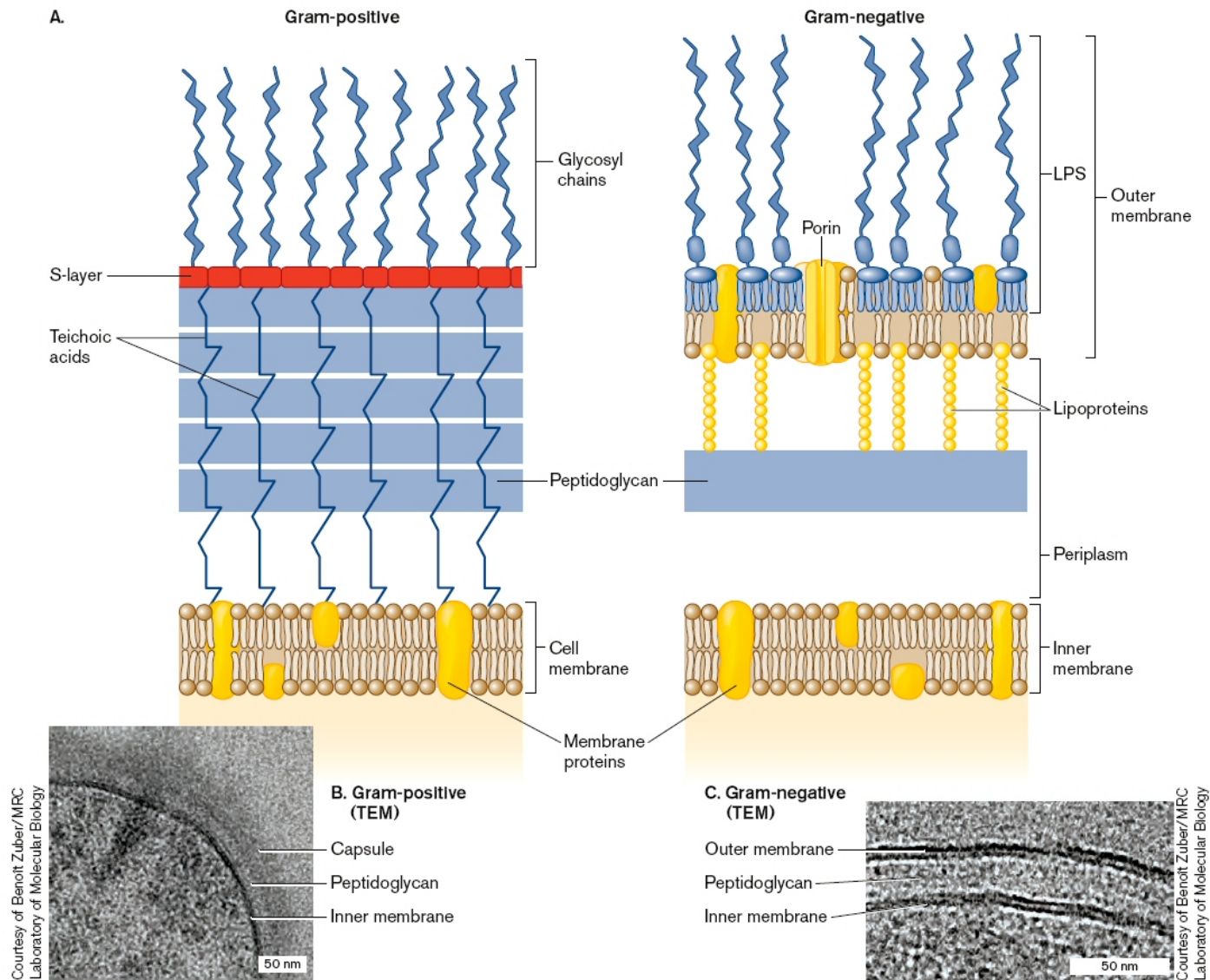


General Bacterial cell biology

- Compact genome (nucleoid: chromosomal DNA wrapped around binding proteins)
- Abundant ribosomes
- Envelope (differs by cell type)
- Different types of flagella (polar, peritrichous)
- REMARKABLE metabolism!!!



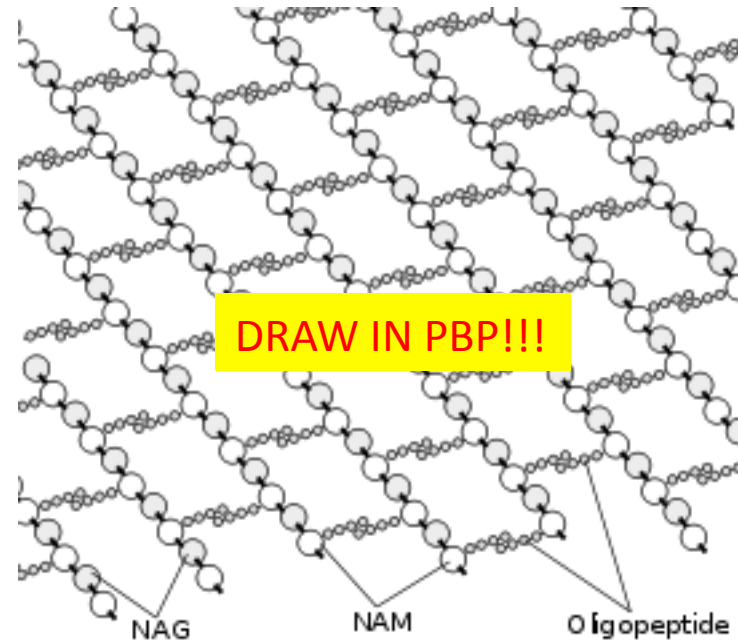
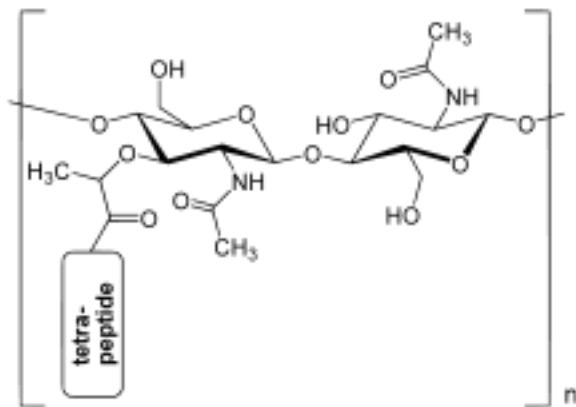
Bacterial cell biology – Gram positive v. Gram negative



Bacterial cell biology –peptidoglycan cell wall

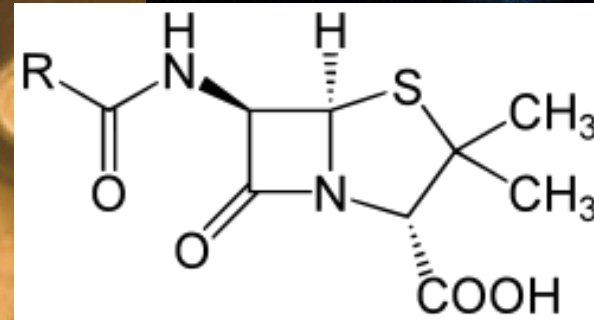
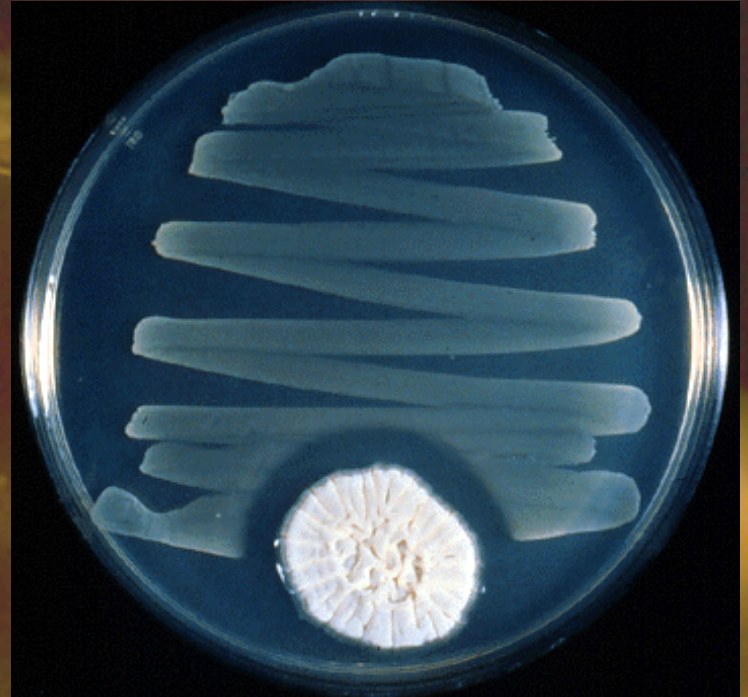
N-acetylglucosamine (GlcNAc or NAG) +
N-acetylmuramic acid (MurNAc or NAM)

NAM attaches to tetra-peptide



Transpeptidases (i.e. (Penicillin binding proteins) catalyze joining of strands

Soil



Natural product pharmaceuticals
29 – penicillin (A. Fleming) – bind transpeptidases

General Archaeal cell biology

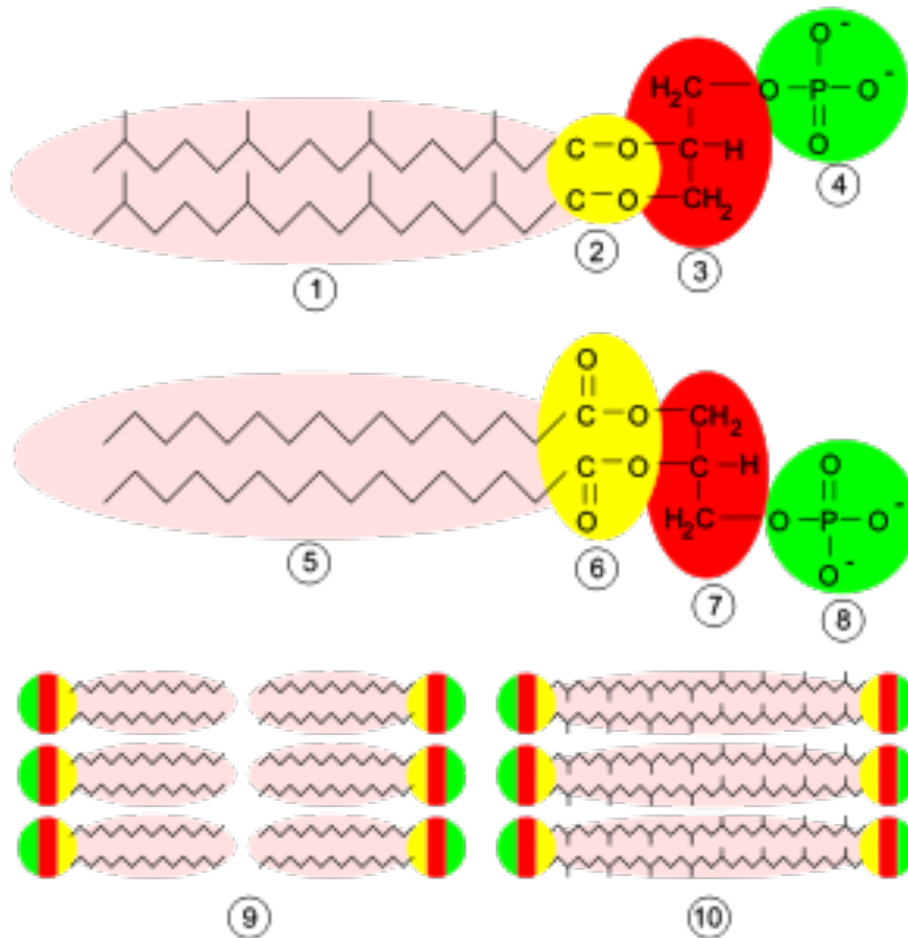
- Compact genome (+ supercoiling by “reverse gyrase”: enhances DNA stab)
- Abundant ribosomes (machinery closer to eukarya than bacteria – Week 6)
- Envelope (structurally different)
- Specialized types of metabolism

Pyrococcus furiosus (“rushing fireball”)
*Isolated in deep sea vents off Italian coast by
Karl Stetter*



Hyperthermophile: grows 70 – 103 C

Archaeal cell biology—cell membrane



Membrane structures. **Top**, an archaeal phospholipid: **1**, isoprene chains; **2**, ether linkages (chemically more resistant than ester bonds); **3**, L-glycerol moiety; **4**, phosphate group.

Middle, a bacterial or eukaryotic phospholipid: **5**, fatty acid chains; **6**, ester linkages; **7**, D-glycerol moiety; **8**, phosphate group.

Bottom: **9**, lipid bilayer of bacteria and eukaryotes; **10**, lipid monolayer of some archaea (thought to make more Rigid membranes; adapted to high Temp/low pH).

cell wall = S-layer (crystalline layer of proteins/glycoproteins) also in Gm⁺ bacteria

Can cells transport things by diffusion alone?

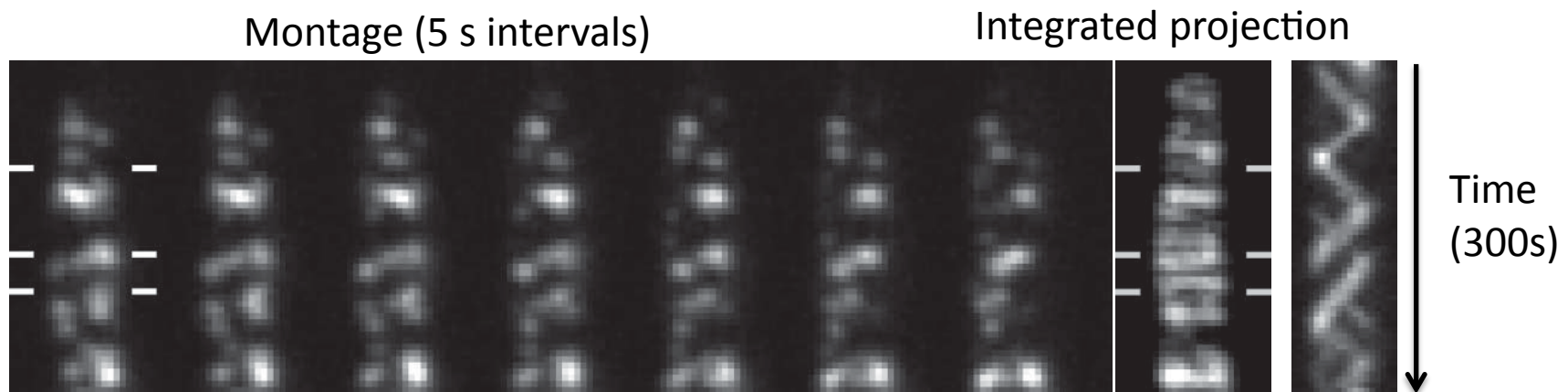
Calculation of time for O₂ to diffuse across a neuron

Cell dynamics: targeted movement of proteins within cells

Example: Dynamic MreB movement (peptidoglycan biosynthesis) in *Bacillus subtilis*
MreB = actin homolog; actin is used in eukaryotic cells for many purposes, including cell motility, mechanical support, scaffold for proteins involved in muscle contraction, transport cargo (vesicles, organelles) within eukaryotic cell

MreB = protein that directs the synthesis and insertion of new peptidoglycan building units

- *For a bacterial cell to divide, more than 10^6 peptidoglycan subunits must be attached to existing subunits!*
- *Circumferential movement that inserts new radial hoops*

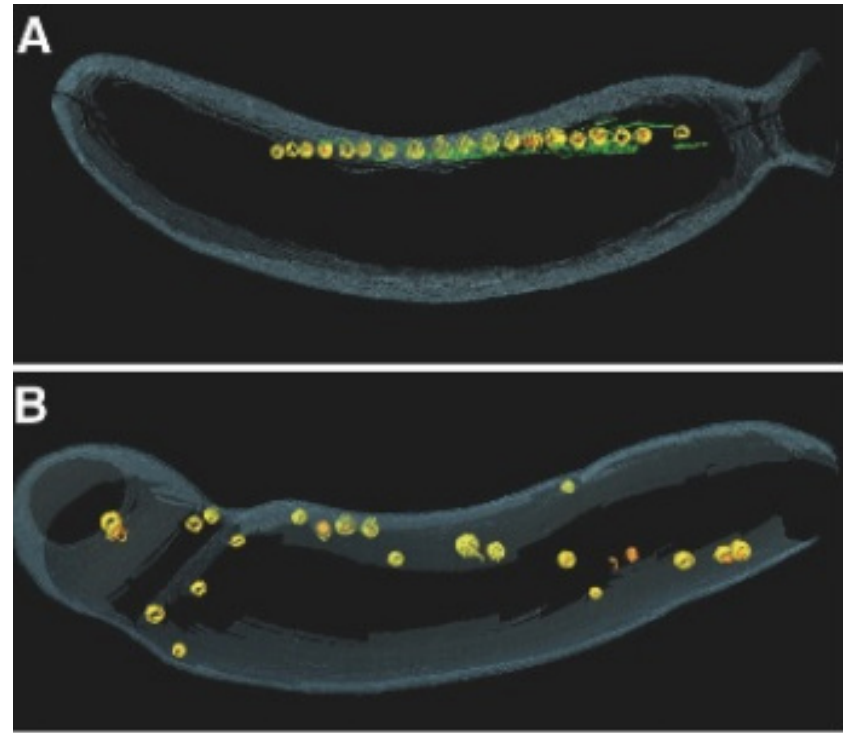


Garner *et al.* (2011) *Science*

Dominguez-Escobar *et al.* (2011) *Science*

Compartmentalization

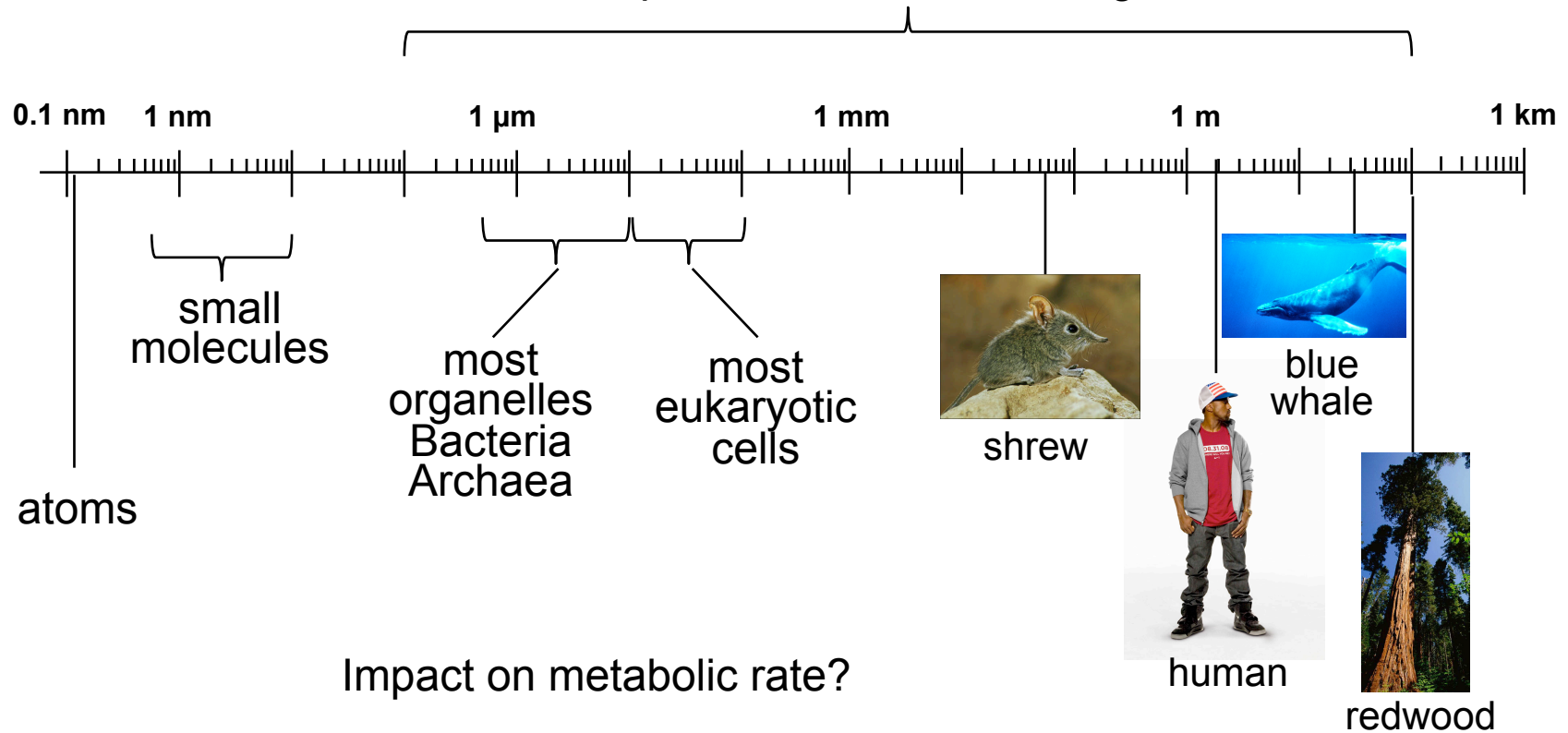
- Magnetosomes
- Photosynthetic membranes (vesicles, lamella)
- Carboxysomes (surrounded by a protein coat), concentrate RuBISCO
- Polyphosphate
- Much still to discover!
- How do they form? Why do they form?
-



Key concepts for today – Part 2:

1. Origin of the eukaryotic cell
2. Multicellularity – largely about cooperativity

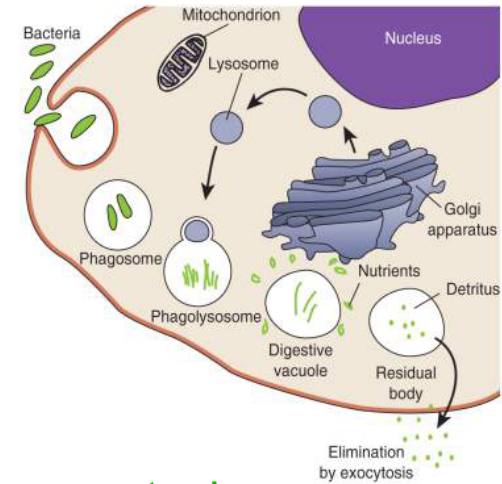
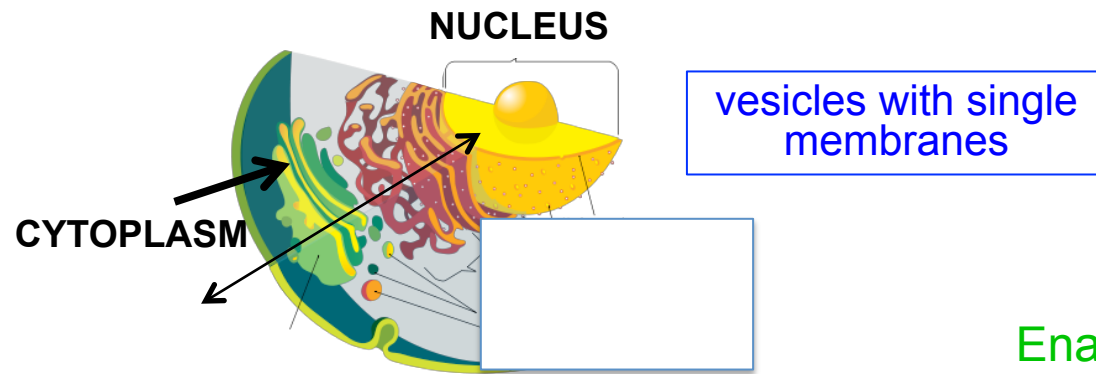
Life forms span over 9 orders of magnitude in size



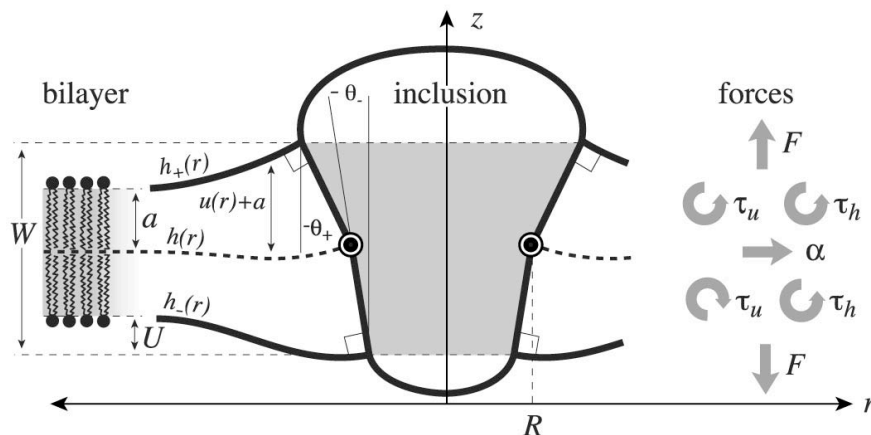
The Eukaryotic Cell

Abundant infolding produces complex network of intracellular membranes and vesicles.

Nucleus – houses cell's DNA
Present in most cells –
not RBCs/lens fiber cells
usually 1, sometimes more



Enables phagocytosis
(Lost in plants/fungi)

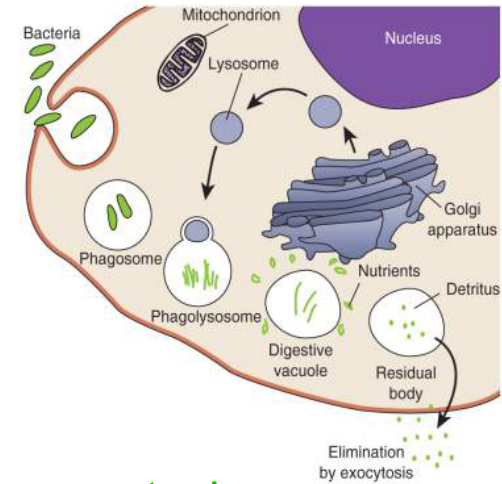
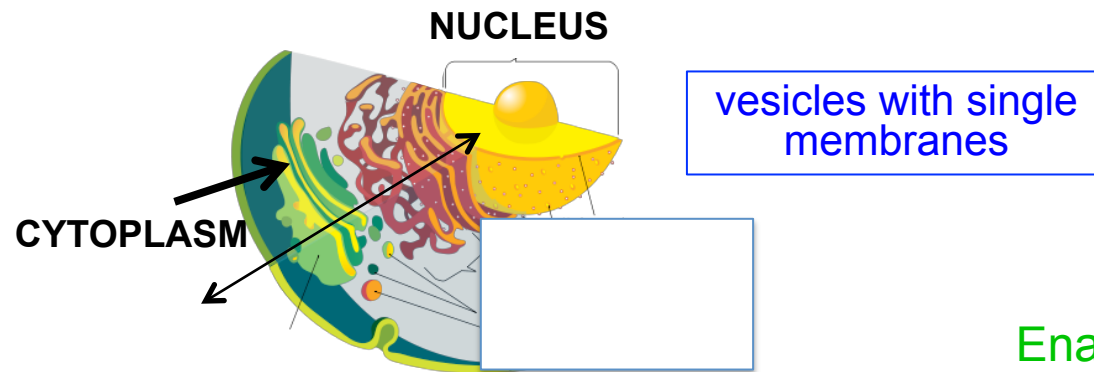


Rob Phillips – Applied Physics

The Eukaryotic Cell

Abundant infolding produces complex network of intracellular membranes and vesicles.

Nucleus – houses cell's DNA
Present in most cells –
not RBCs/lens fiber cells
usually 1, sometimes more

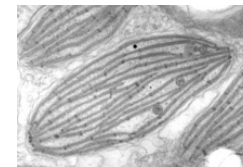


Enables phagocytosis

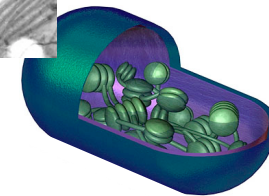
Features with double membranes and...nuclei acids, lacking histones, bacteria- specific protein complexes:



MITOCHONDRIA

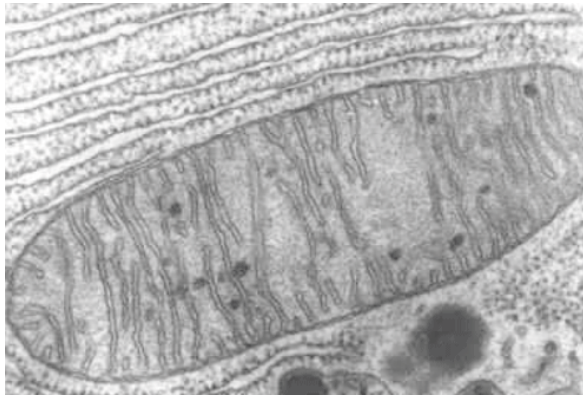


PLASTIDS

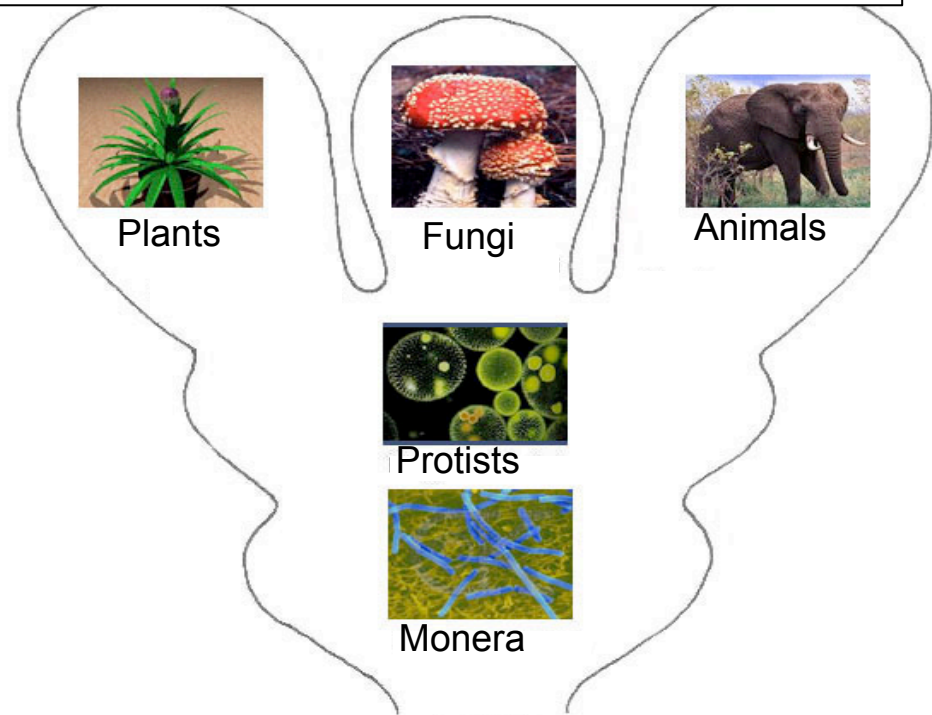


BACTERIAL in ORIGIN?

Endosymbiosis Theory of the Origin of the Eukaryotic Cell



Lynn Margulis 1938-2011



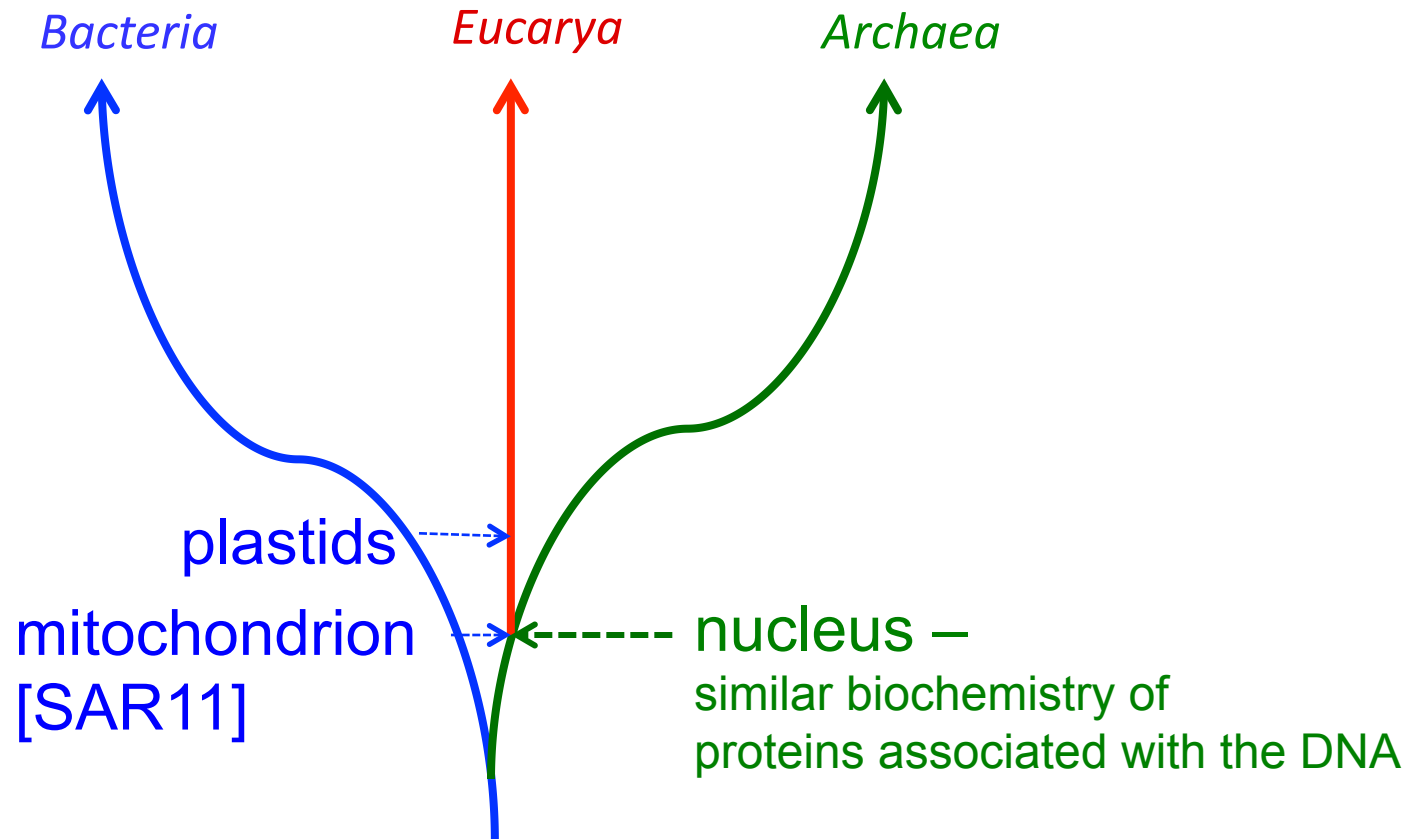
Five Kingdom Model

L. Sagan (1967) On the origin of mitosing cells
Journal of Theoretical Biology

Married to Carl Sagan

Manuscript rejected by over a dozen journals

Observations led to short-lived 5-kingdom model
Endosymbiosis theory lives on



The Eukaryotic Cell

The mitochondrion:

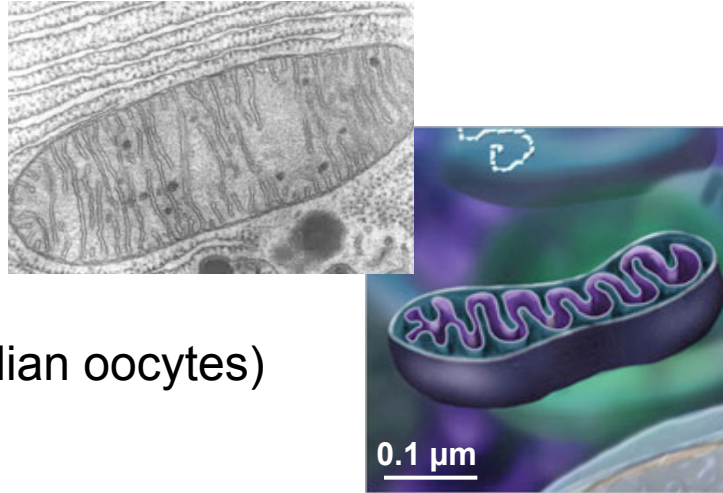
0 – 100,000/cell (mammalian oocytes)

Two principal functions:

1. oxidative phosphorylation – generation of ATP –
canalized – only use of O_2 as terminal electron acceptor
organics + O_2 to CO_2 + H_2O + ATP
2. biosynthesis of Fe-S clusters* only essential function of mito – *only*
happens in mito, except when mito absent (NUCLEAR);
 - electron transfer (redox potentials from -500 to +300 mV, so can
act as acceptors or donors)
 - enzyme cofactor
 - sensing environment or intracellular state to change gene expression

associated diseases (colon cancer to types of anemia to myopathy)

Maternal inheritance, a few get in with sperm, but they're destroyed, mitochondrial Eve



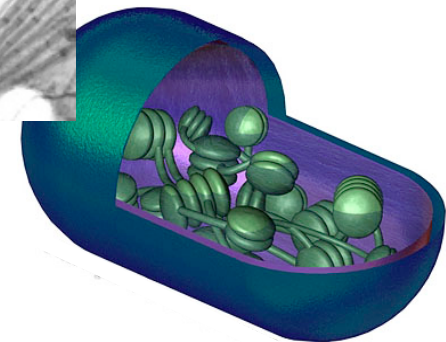
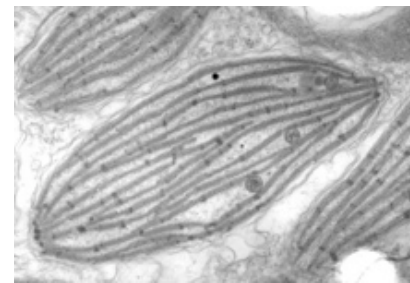
Plastids organelles in algae and plants:

Several types, e.g., different functions in different plant tissues
[won't give names, just functions]

- *1. chloroplasts – carry out photosynthesis (primarily leaves)
- 2. amyloplasts – for bulk storage of starch (roots)
- 3. chromoplasts – pigment that provide color (petals)

in PS * $\text{CO}_2 + \text{H}_2\text{O} + h\nu$ to organic C + O_2

UNLIKE IN BACTERIA, PS always requires H and C in eukaryotes restricted to
- canalized to use H_2O and CO_2

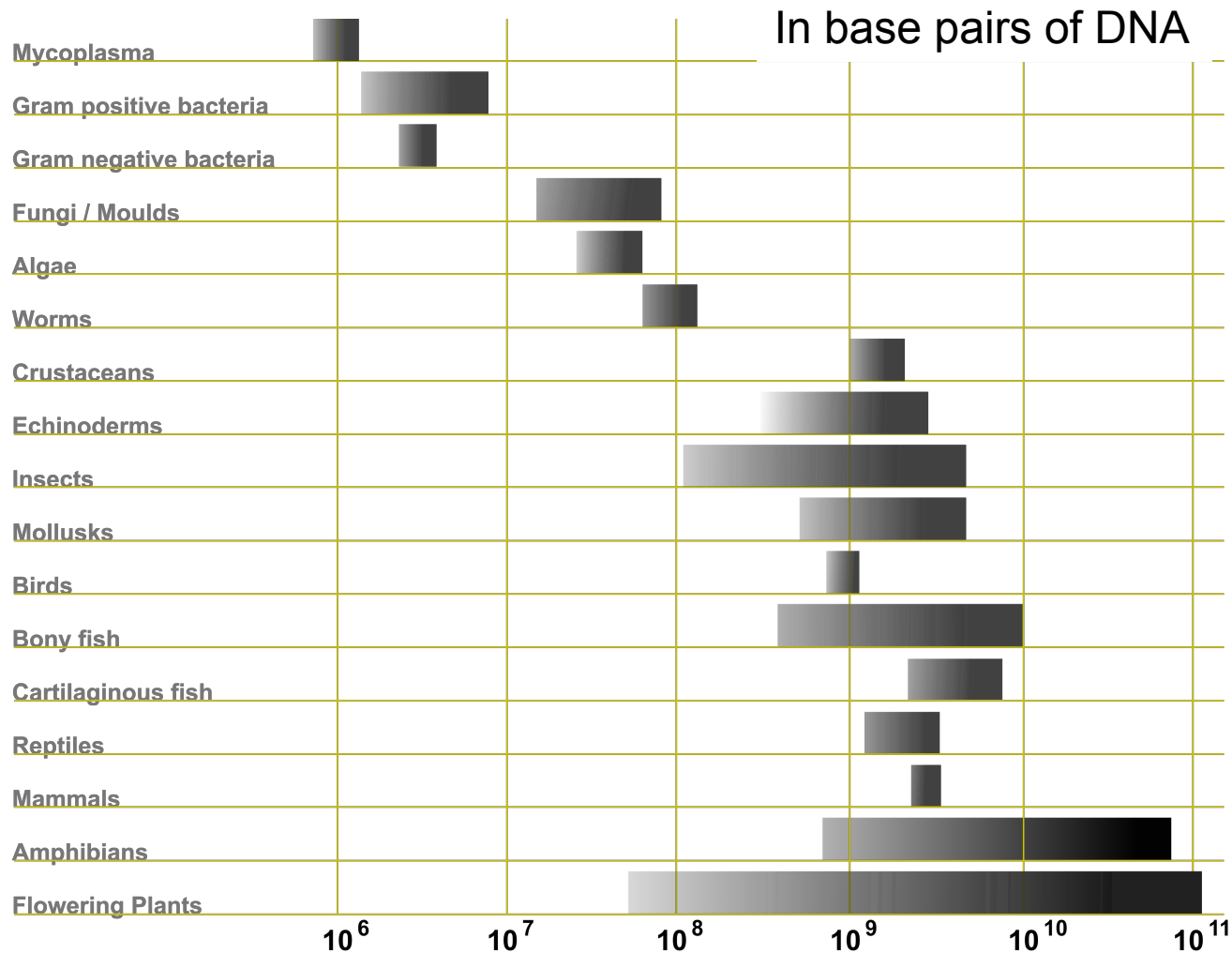


PLASTIDS

OTHER MAJOR DIFFERENCES THAT ARISE WITH THE EUKARYOTES

- differ in arrangement of genes, genes on series of discrete linear chromosomes, have large non-coding regions between each gene transcribe the DNA differently
[Bacteria and Archaea – highly compact and metabolically efficient]
- larger genome size

Eukaryotic cells have larger genome sizes



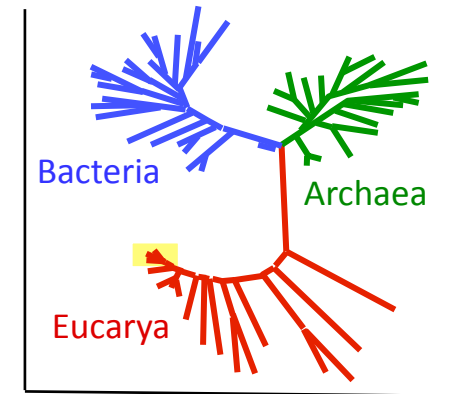
Smallest 160 kb (a symbiotic bacterium)

Largest 670 Gb – an amoeba (protist)

Human, 3.2 Gb; lungfish 130 Gb

Multicellularity

biosphere



BACTERIA/ARCHAEA
(Usually single-celled?)

EUKARYA

Single-celled
(USUALLY)

Multicellular
(plants/animals/fungi)
(USUALLY)

ecosystem

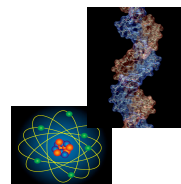
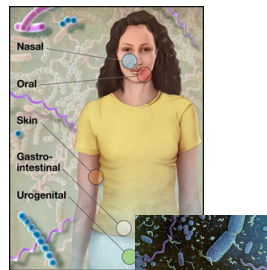
community

population

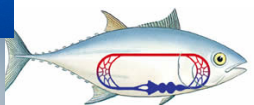
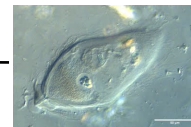
organism
[cell]

molecule

atom

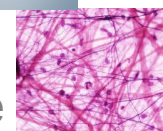


organelle



organ

tissue



organ system

Multicellularity

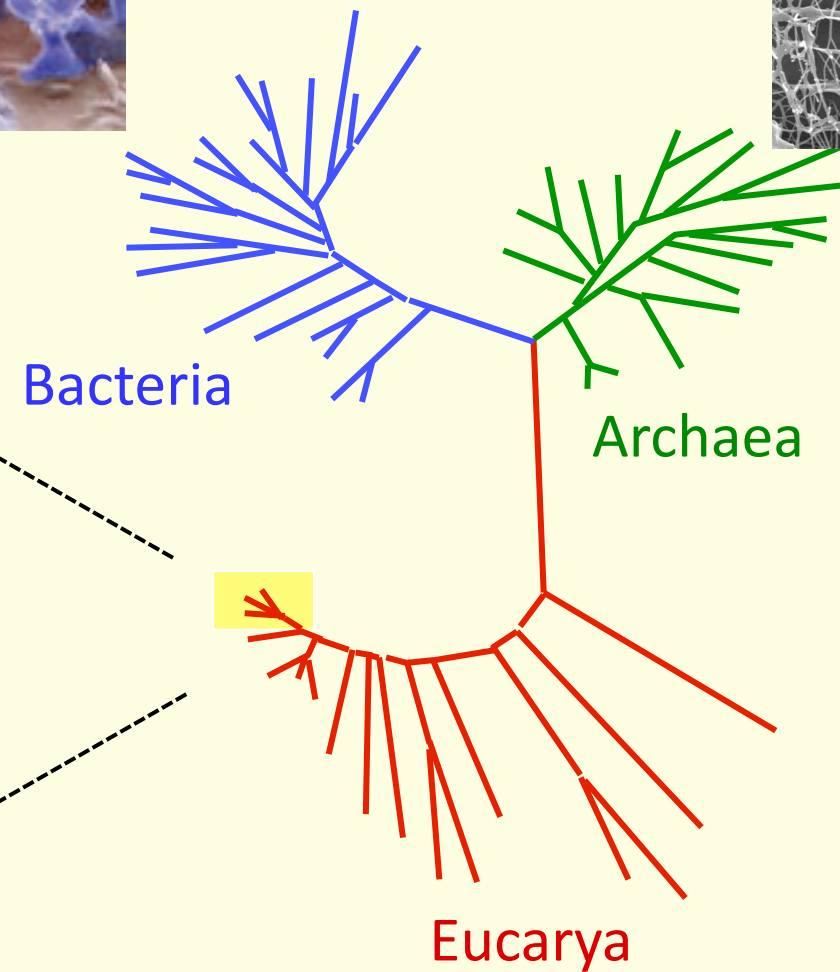
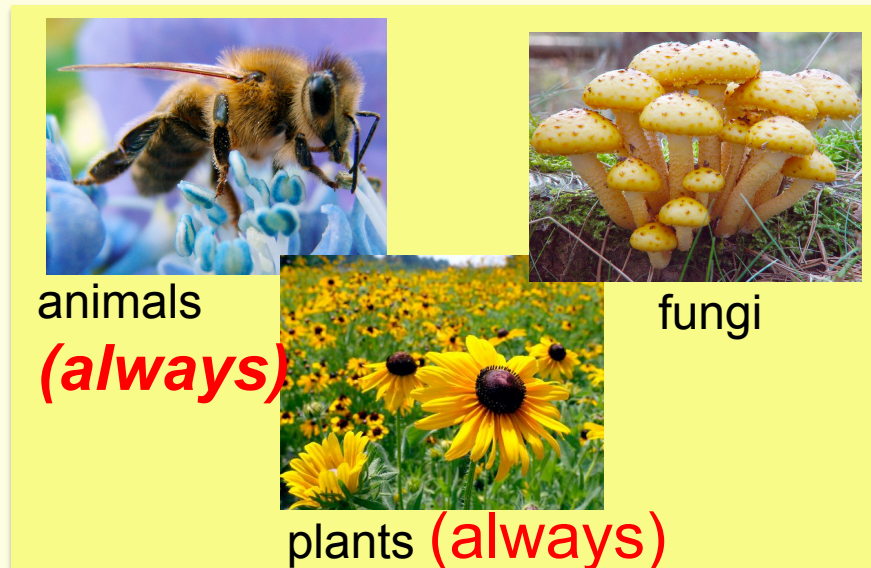
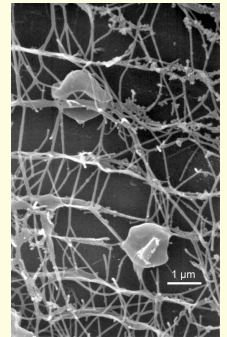
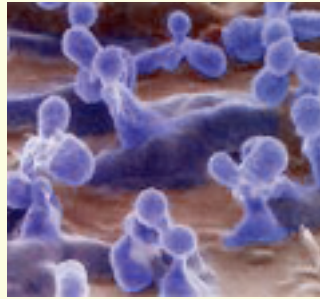
Groups of cells +/- differentiation (division of labor)

Arises by one of two mechanisms:

- 1) Cell aggregation
- 2) Lack of separation on division

ω is greater with multicellularity
if selection favors cooperation.
If selection favors conflict not so.

Multicellularity occurs in representatives of all three domains



Difference in complexity

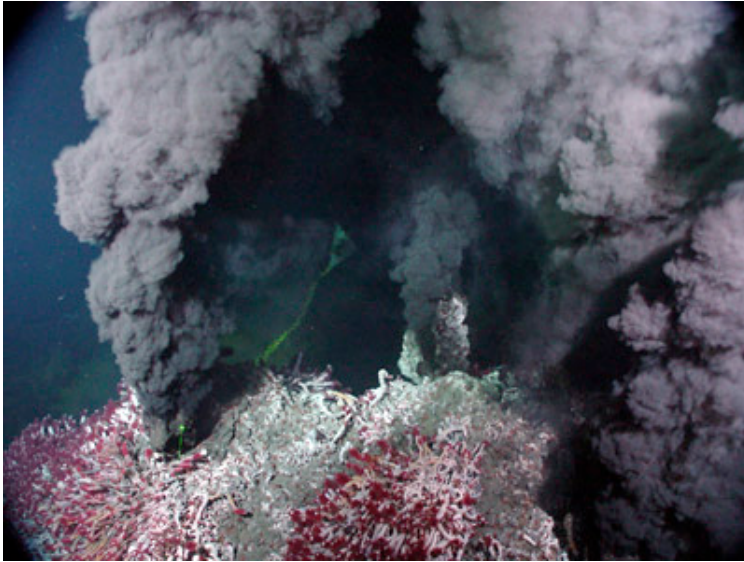
Bacteria – 2-3 cell types

Fungi – seven-nine cell types

Plant/animal tens of different cell types

(humans have hundreds of cell types)

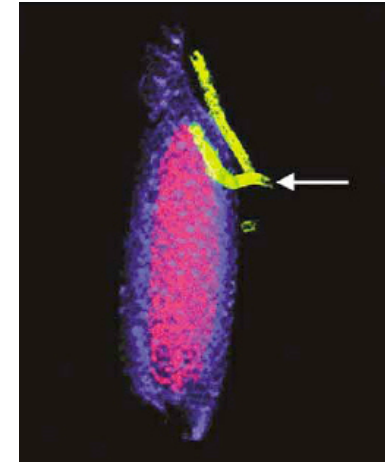
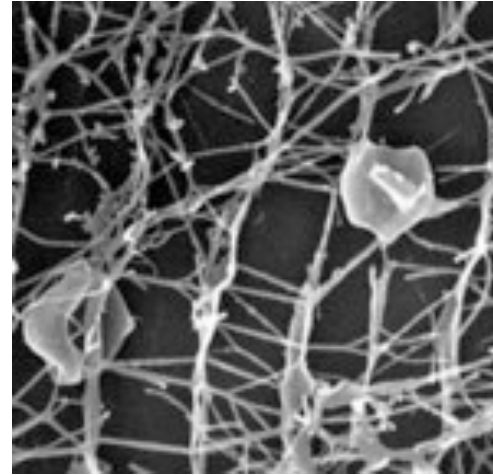
Multicellular Archaea



Hydrothermal vent, mid-Atlantic
ridge, deep sea (~3000 m)

Pyrodictium oculatum

Optimal growth 80 – 105 degrees C

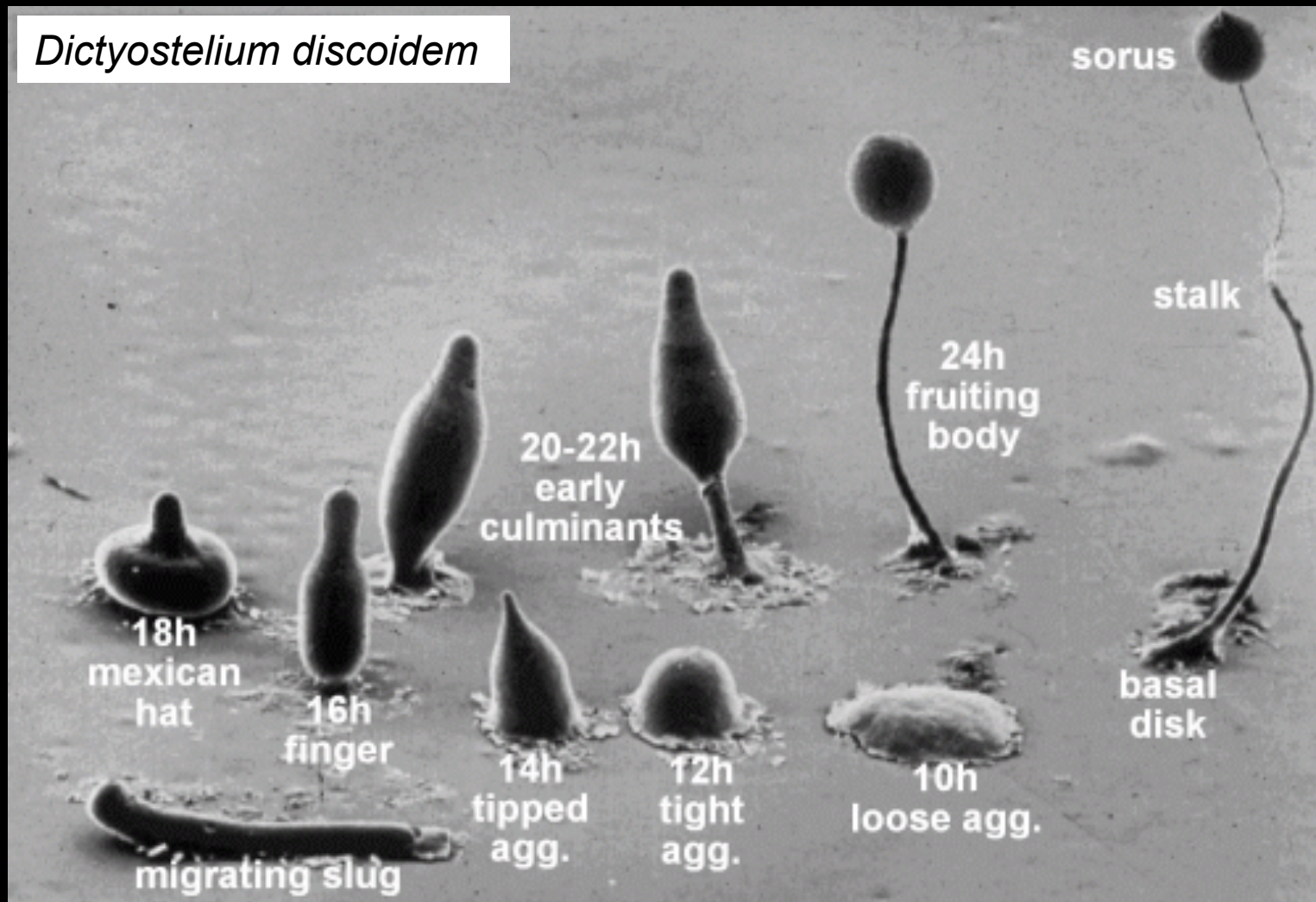


Dictyostelium discoideum

**Soil-living amoeba, solitary when
food abundant**

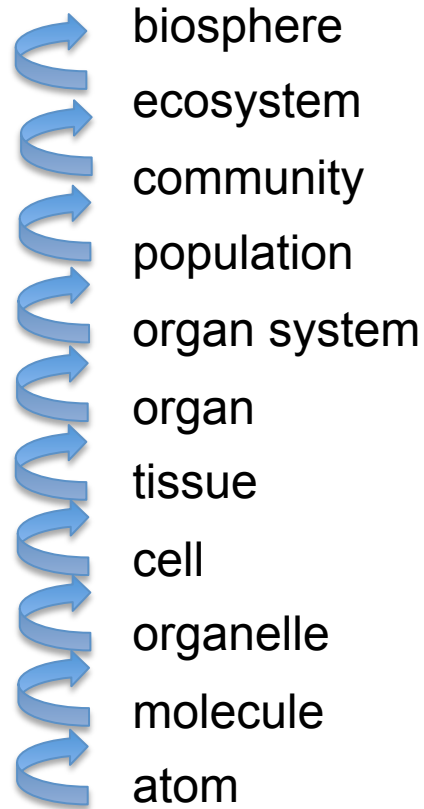
MOVIE

Dictyostelium discoideum

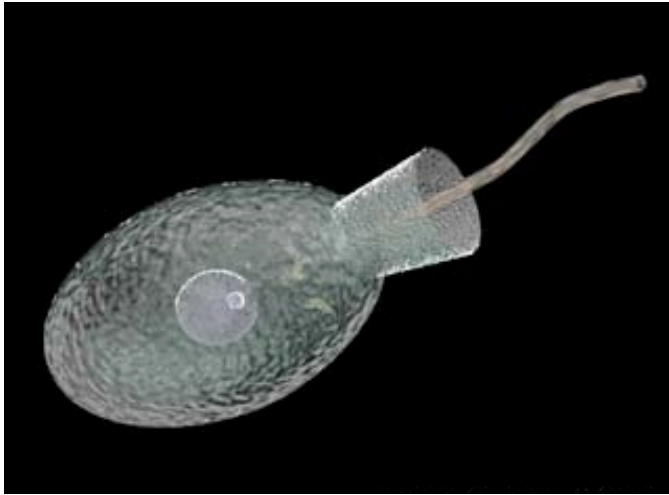


Differentiation, division of labor, self

Where in the hierarchy? What emergent properties?

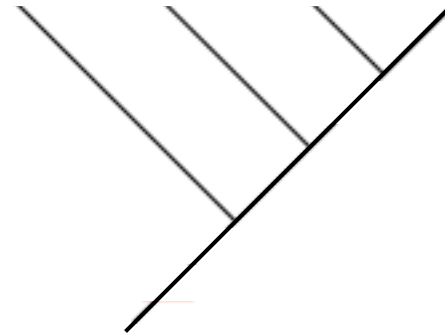


Obligately multicellular organisms – e.g., animals



Choanoflagellates – Sister group to the animals

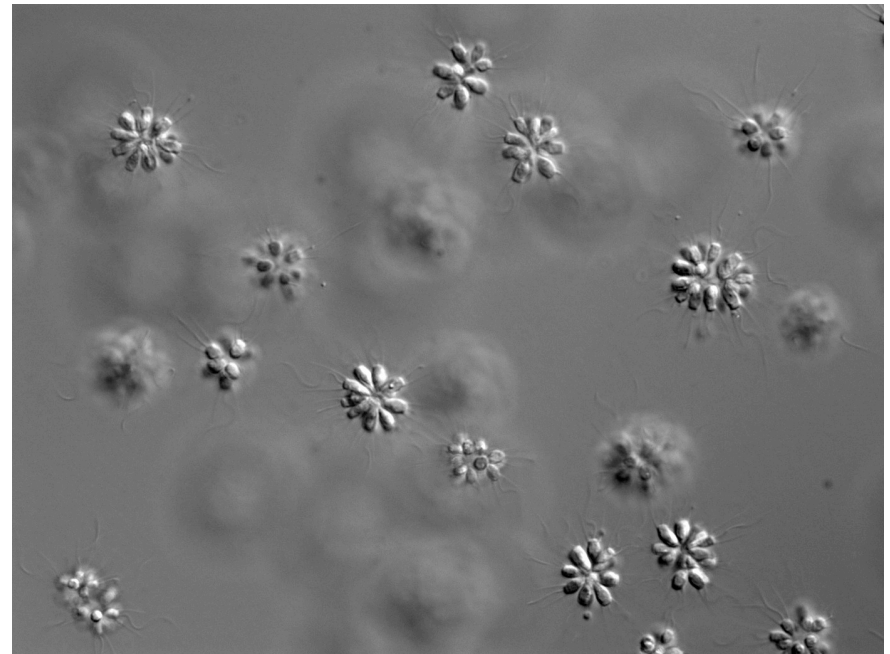
Plants Fungi Choanos Animals



Become multicellular under the influence of specific **bacteria** –

What about the bacteria induce this behavior?

BACTERIAL SPHINGOLIPIDS
RARE membrane components
in *Bacteroides* - signaling



What pre-adapted the choanoflagellates to respond?

Although often unicellular choanoflagellates have proteins that enable **cell-cell adhesion** and **signaling**.

Including:

cadherins – prey capture

tyrosine kinases

