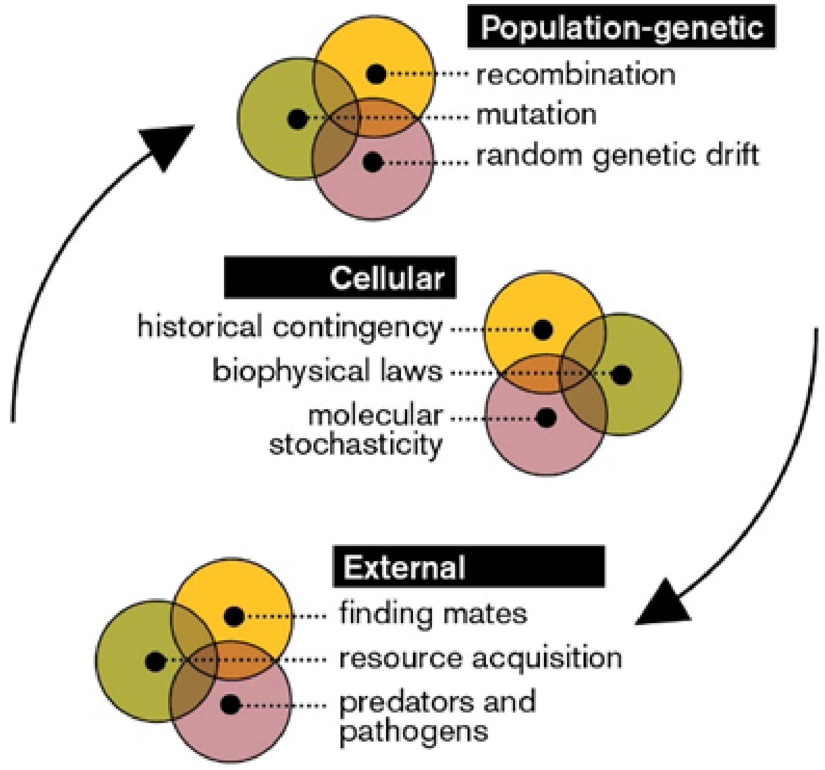
The Cellular Environment

- How do the basic structures and contents of cells constrain the paths down which evolution can proceed?

- Historical contingencies:
    - Elemental composition and environmental availability.
    - Carbon and energy requirements for cell replication.

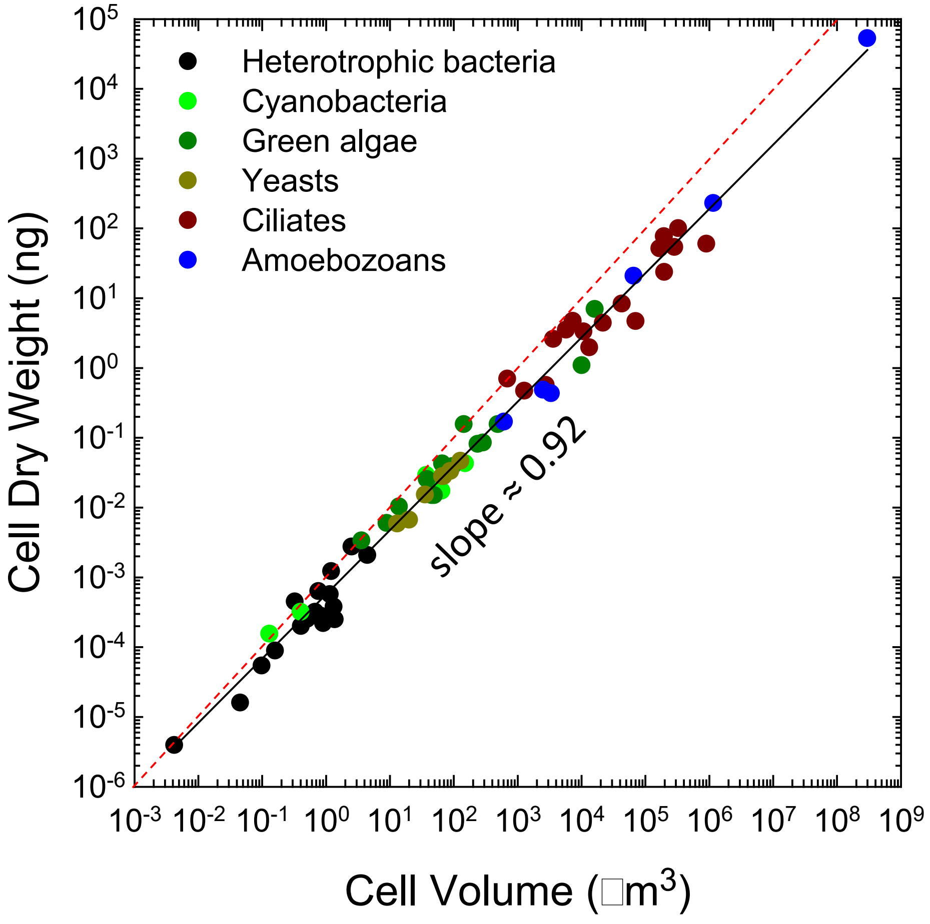
- Biophysical constraints:
    - The special properties of water.
    - Diffusion of intracellular constituents.
    - Temperature.

- Molecular stochasticity:
    - Numbers of molecules per cell and stochastic inheritance.



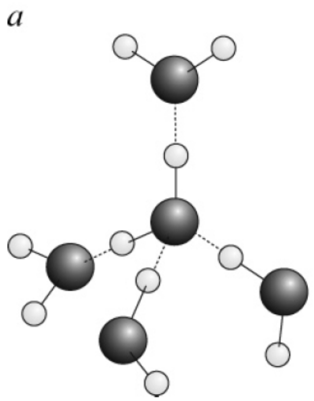
Three Aspects of the Environment Relevant to Evolutionary Processes

Universal Scaling of the Water Content of Cells

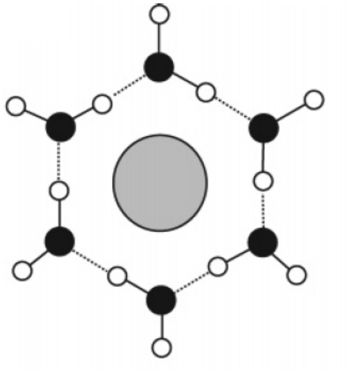


- Between 1/5th and 1/3rd of cell wet weight of consists of H_2O, eukaryotic cells being more watery than those of bacteria.

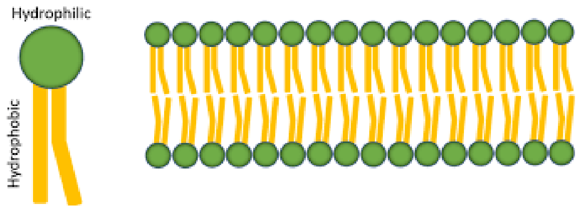
The Special Features of Water



- The unique physical properties of water govern almost every aspect of biology:
  - the folding stability of proteins,
  - the ability of lipid molecules to aggregate into membranes,
  - the diffusion rates of molecules,
  - the challenges to motility.



- On average, the number of hydrogen-bonded nearest neighbors in bulk water is ~3.5.
  - In order to "stay organized" water builds a cage around soluble particles.
  - Exclusion of nonpolar molecules facilitates the spontaneous assembly of membranes, as the hydrophobic tails of lipid molecules naturally aggregate in a highly coordinated fashion.

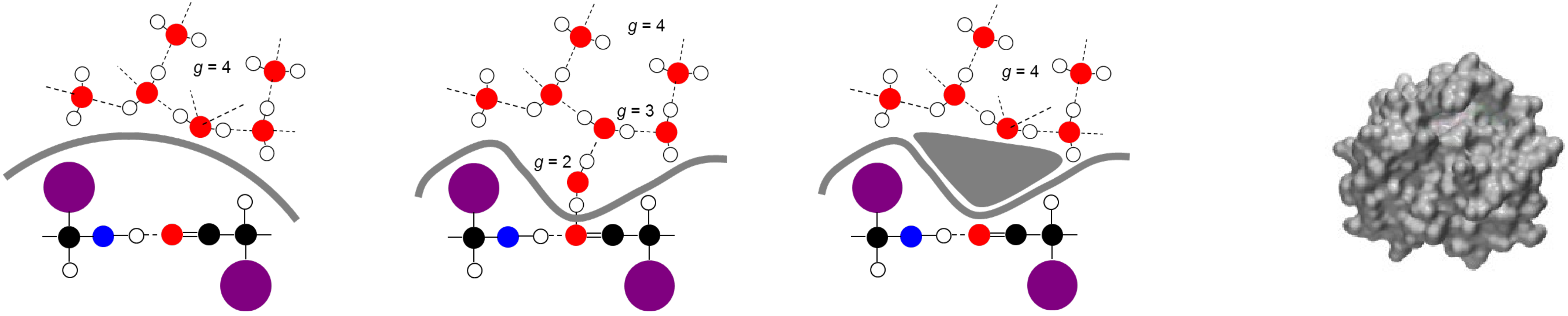


Chem. Rev. **2008**, *108*, 74–108

Water as an Active Constituent in Cell Biology

Philip Ball*

To maintain functionality, proteins need to maintain proper folds and minimize exposure of backbone hydrogen bonds



Well-wrapped   Exposed   Tension relieved

- The inner hydrophobic cores that maintain protein structure can be compromised by the intrusion of water molecules, imposing strong selective pressure for soluble proteins to achieve globular structures by populating their outer surfaces with hydrophilic amino acids.

- Exposed backbone hydrogen bonds reduce protein functionality, and increase stickiness and the potential to engage in protein-protein misinteractions.

Elemental Composition of Cells: almost all of life depends on just 20 key chemical elements.

Species	Size	C	N	P	S	K	Na	Mg	Ca	Cl
Means		11503	1494	115	124	167	255	106	232	429
Seawater		2.25	0.03	0.002	28	10.2	469	52.7	10.3	546
Cellular enrichment		5,100	50,000	57557	4.4	16.4	0.5	2.0	22.6	0.8

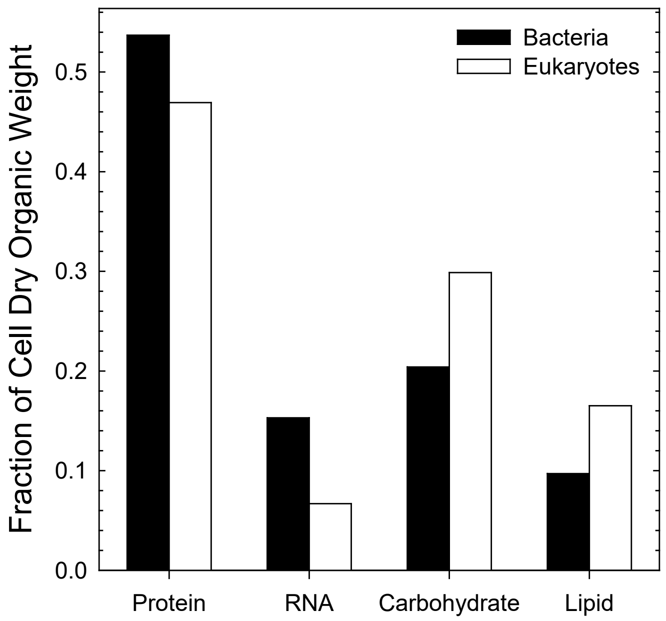
mM concentration

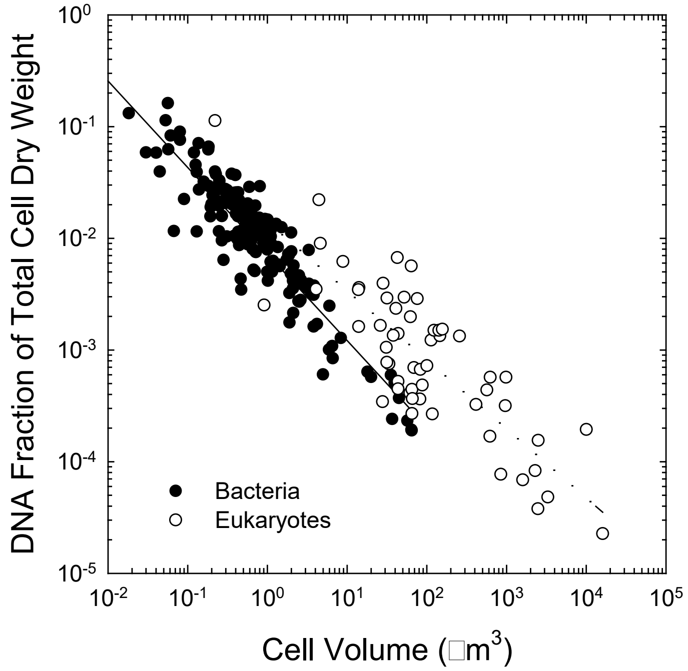
	Sr	Fe	Mn	Zn	Cu	Co
Means	796	680	395	129	62	24
Seawater	89	0.00054	0.00036	0.0054	0.0024	0.000020
Cellular enrichment	8.9	1,260,000	1,086,000	24,000	26,000	1,182,000

µM concentration

- Many of the chemical elements essential to life have intracellular concentrations enriched by factors of 10^3 to 10^6 relative to environmental levels.
- Factors = volume of the environment relative to cell volume that needs to be fully harvested to produce an offspring cell.

Biomolecular Composition of Cells

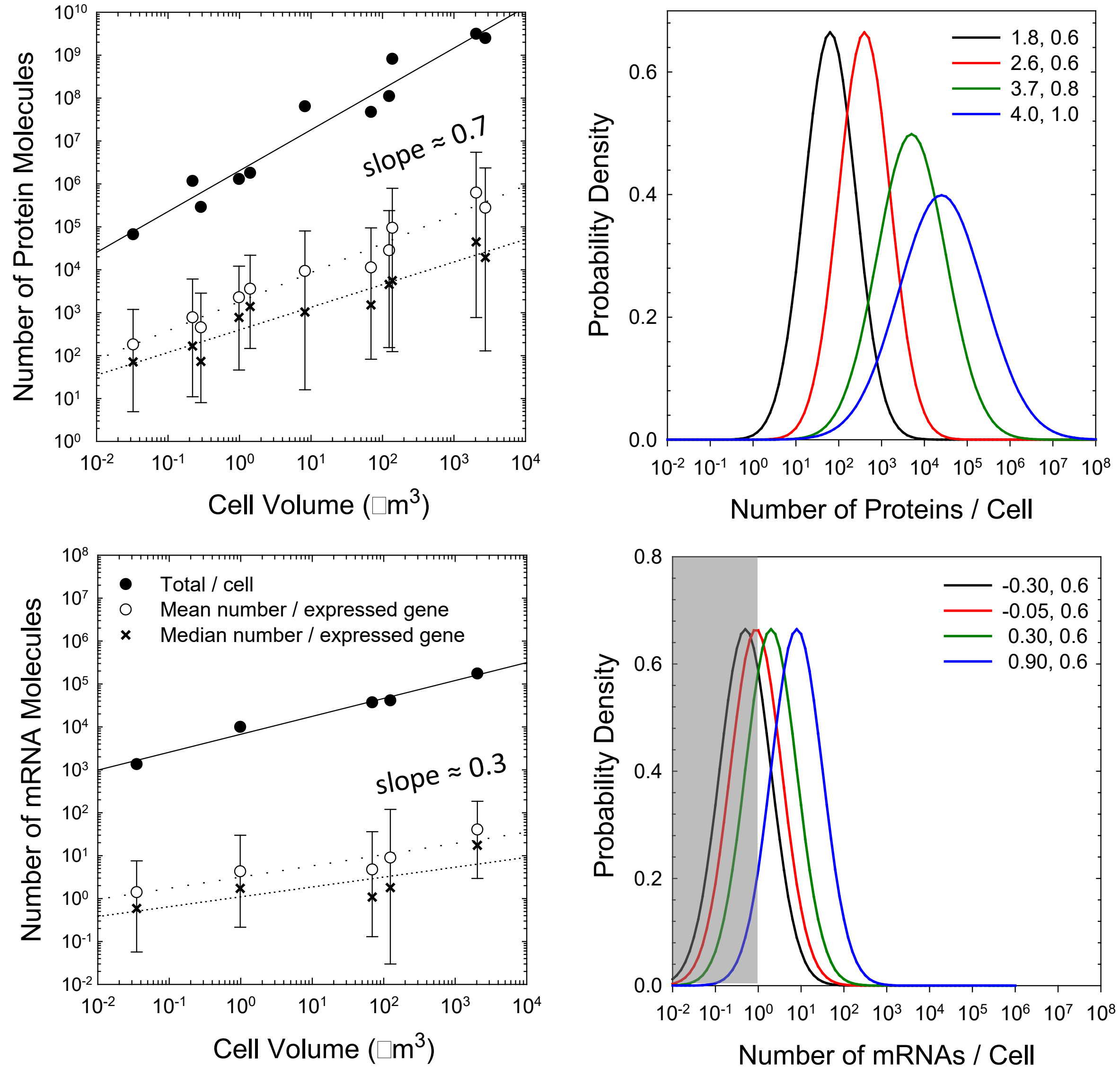




- Across the Tree of Life ~50% of cell dry weight consists of carbon atoms, and ~50% of dry weight is protein.

- Species with larger cells tend to have larger genomes, but the proportional contribution of DNA declines.

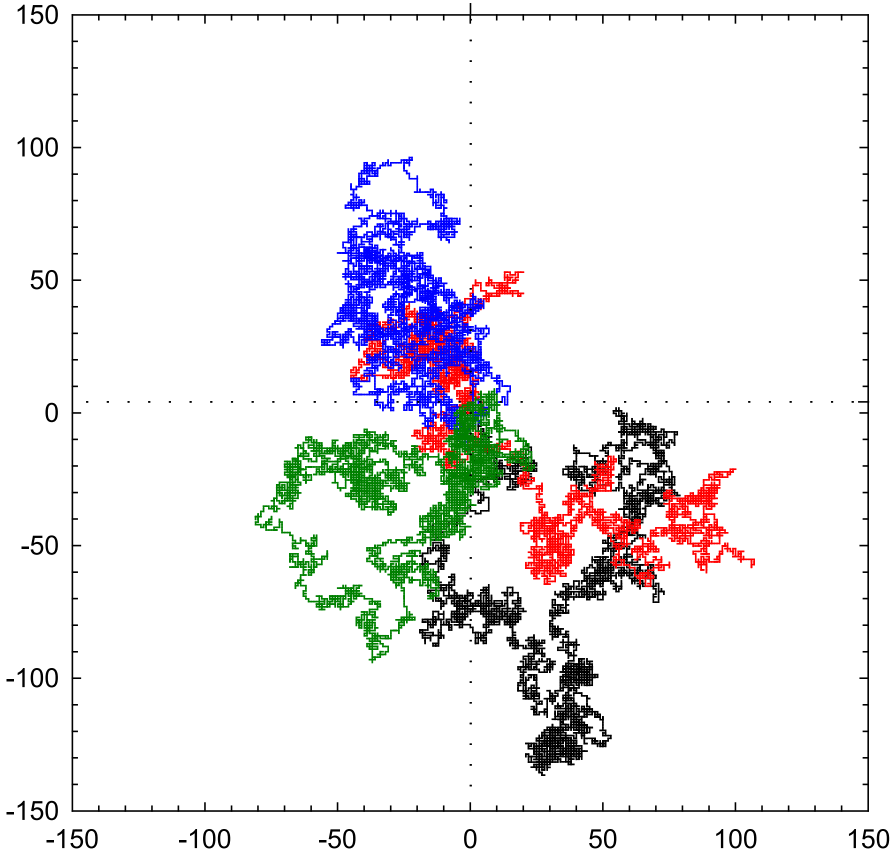
Numbers of Biomolecules per Cell



- Numbers of biomolecules scale sublinearly with cell volumes across the Tree of Life.
- Scaling is much stronger for protein numbers than for messenger RNA numbers.
- Messenger RNAs are typically 100- to 10^4-fold less abundant per cell than their cognate proteins.
- Molecular stochasticity: in small cells, the number of copies of some proteins can be < 100 / cell, and the numbers of mRNAs / gene can be < 1.

Legend (right): *Mycoplasma, E. coli*, yeast, mammal.

Intramolecular Diffusion: many molecules travel through cells by passive diffusion processes, imposing no costs to the cell.



Diffusion coefficient:

$$D = \frac{k_B T}{6\pi \eta r}$$ = thermal jostling rate / resistance to jostling

radius of particle

viscosity of medium

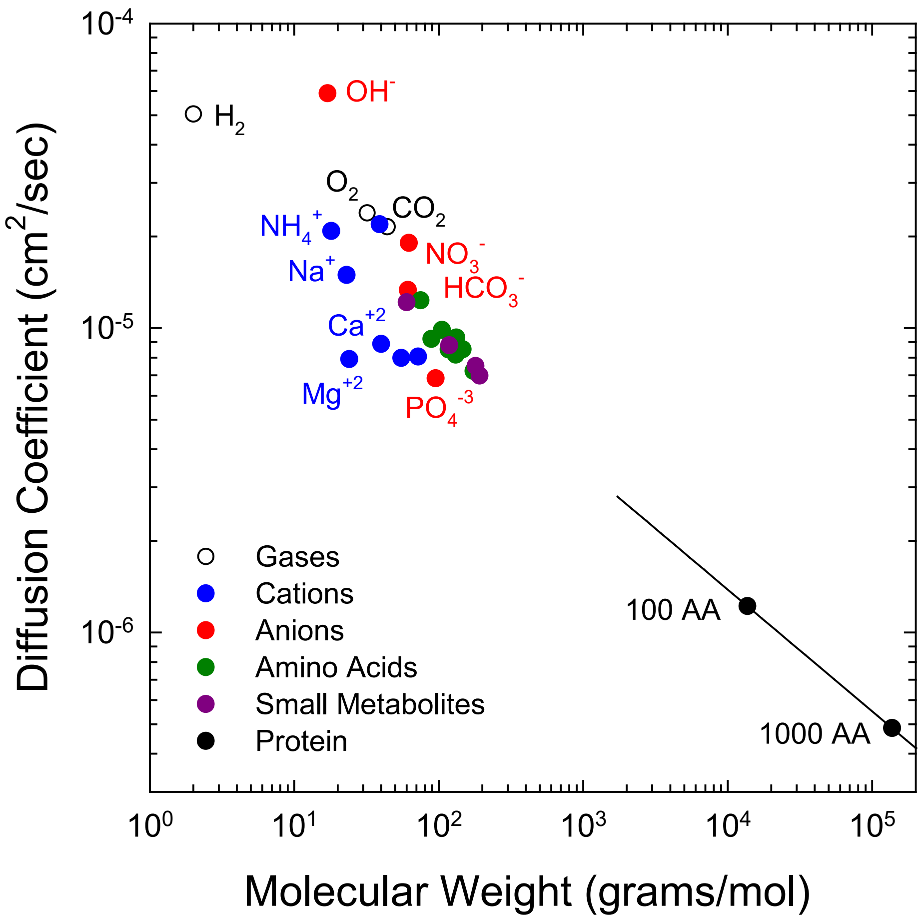
Mean squared distance traveled after t time units:

2Dt in one dimension,
4Dt in two dimensions,
6Dt in three dimensions.

- For small bacteria, an average protein can diffuse across a cell diameter in several milliseconds. In some of the larger eukaryotic cells, such a sojourn can require up to half a minute.

- Thus, diffusion limits to intracellular transactions can ultimately constrain the rates of biological processes in eukaryotic cells.

Diffusion Coefficients for Small Molecules



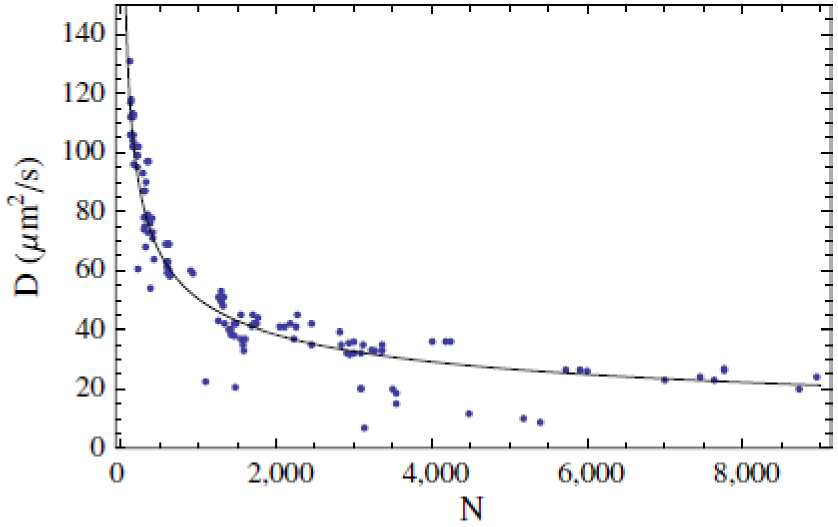
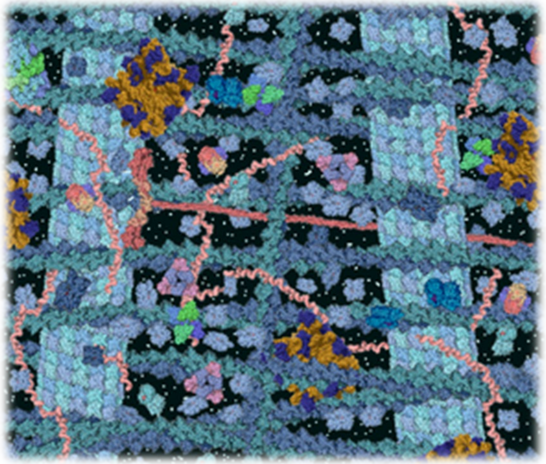


Fig. 5. Dependence of diffusion constant on chain length. Comparison of experimental in vitro diffusion constants (52) to Eq. 9 with the Tyn and Gusek approximation $R_h = 1.45R_n$. Molecular weights have been converted to amino acid numbers using $N = M_w/110$ Da.

- Proteins diffuse more slowly than expected based on mass, owing in part to being nonspherical.

Additional Complications in Biology: Molecular Crowding in the Cytoplasm



- Total volume of cellular fluids occupied by proteins, nucleic acids, and polysaccharides is in the range of 7 to 40%.

- The space between macromolecules is often less than their diameters.

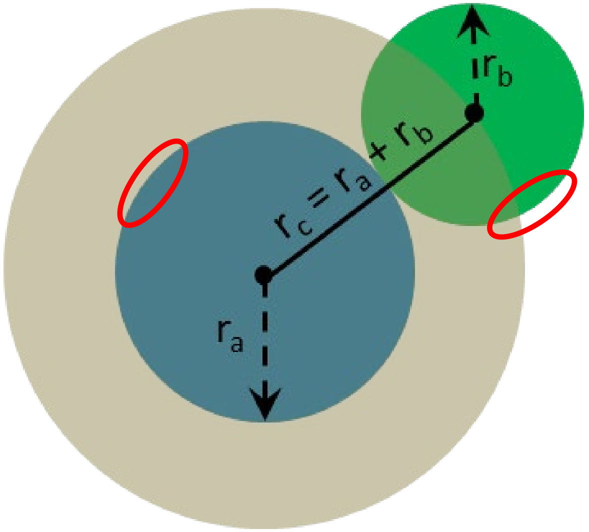
- This reduces rates of diffusion by factors of 5 to 15.

- Relative to prokaryotes, the cytoplasm is somewhat less crowded in eukaryotes, but protein sizes are increased.

- Molecules can be transported by molecular motors.

- Molecular motors can generate background molecular motion.

Encounter Rates Between Particles



- A function of:
  - the two particle concentrations;
  - the summed radii of the two particles;
  - the total rate of molecular movement (the sum of the two diffusion coefficients).
- Additional considerations:
  - attractive / repulsive forces;
  - rotational diffusion to align to particular interacting patches.
- Provides a definition of the biophysical limit to chemical reaction rates.

Temperature and Cellular Functions

- Through its influence on molecular motion, temperature plays a governing role in all reaction rates.
  - Within certain limits, increased temperature generally leads to increased biochemical reaction rates.
  - Beyond certain limits, protein folding stability breaks down.
  - Membrane fluidity increases with increasing temperature.
  - Viscosity increases 50% with a temperature decline from 40 to 4^{o}C, increasing cell buoyancy but decreasing rates of intracellular diffusion and the costs of swimming.

- Dozens of mathematical expressions have been proposed to explain thermal-response behavior.
  - The simplest is the “rule of thumb” that $Q_{10} \approx 2$ to 3.

Arrhenius Rate Behavior

- Letting E_a = the activation energy of a reaction,

$$f_e = e^{-E_a/(k_B T)}$$ is the fraction of molecules above the activation barrier; $f_e \rightarrow 1.0$ as T increases.

- Total reaction rate:

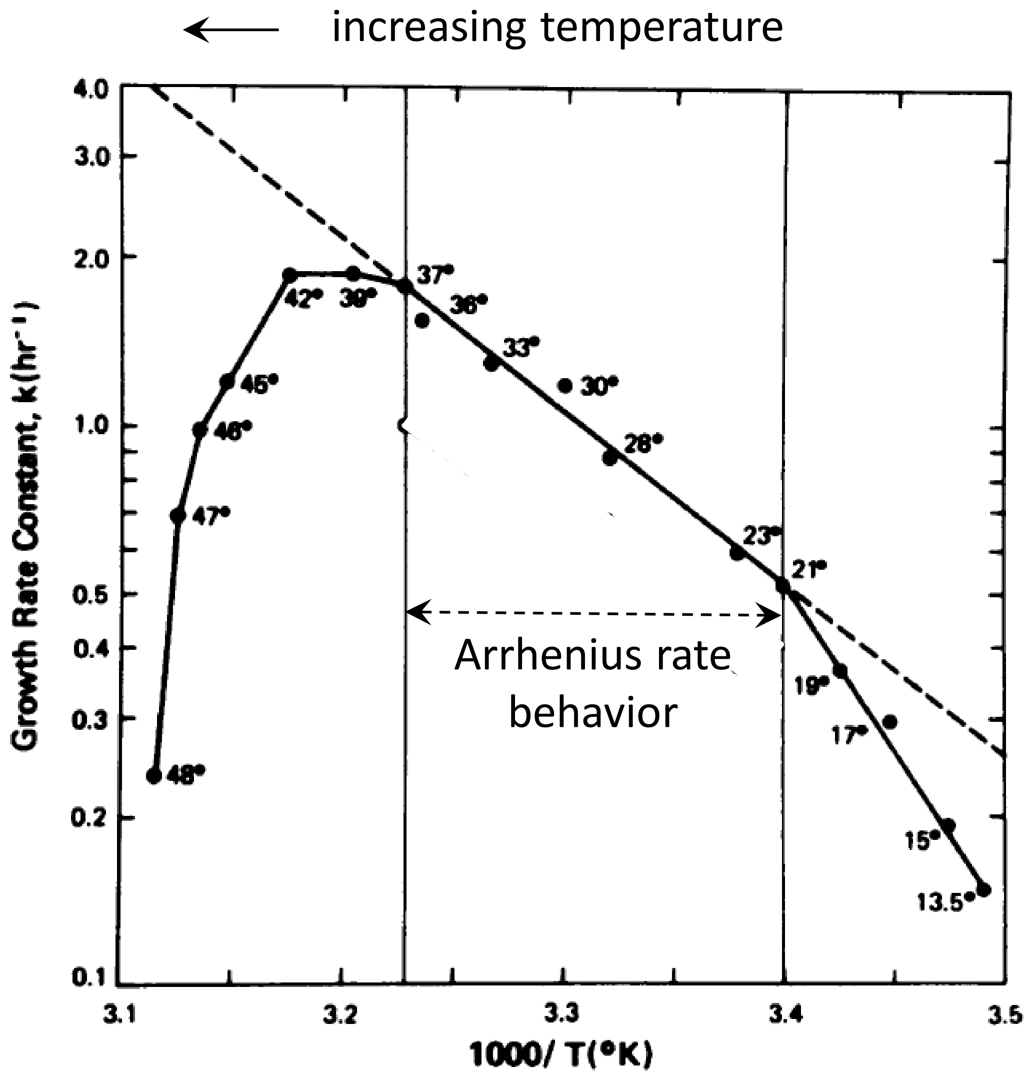
$$R_{\text{tot}} = R_E \cdot f_e = R_E \cdot e^{-E_a/(k_B T)}$$

R_E: encounter rate, a function of reactant concentrations and diffusion rates

- Log transformation of the total reaction rate:

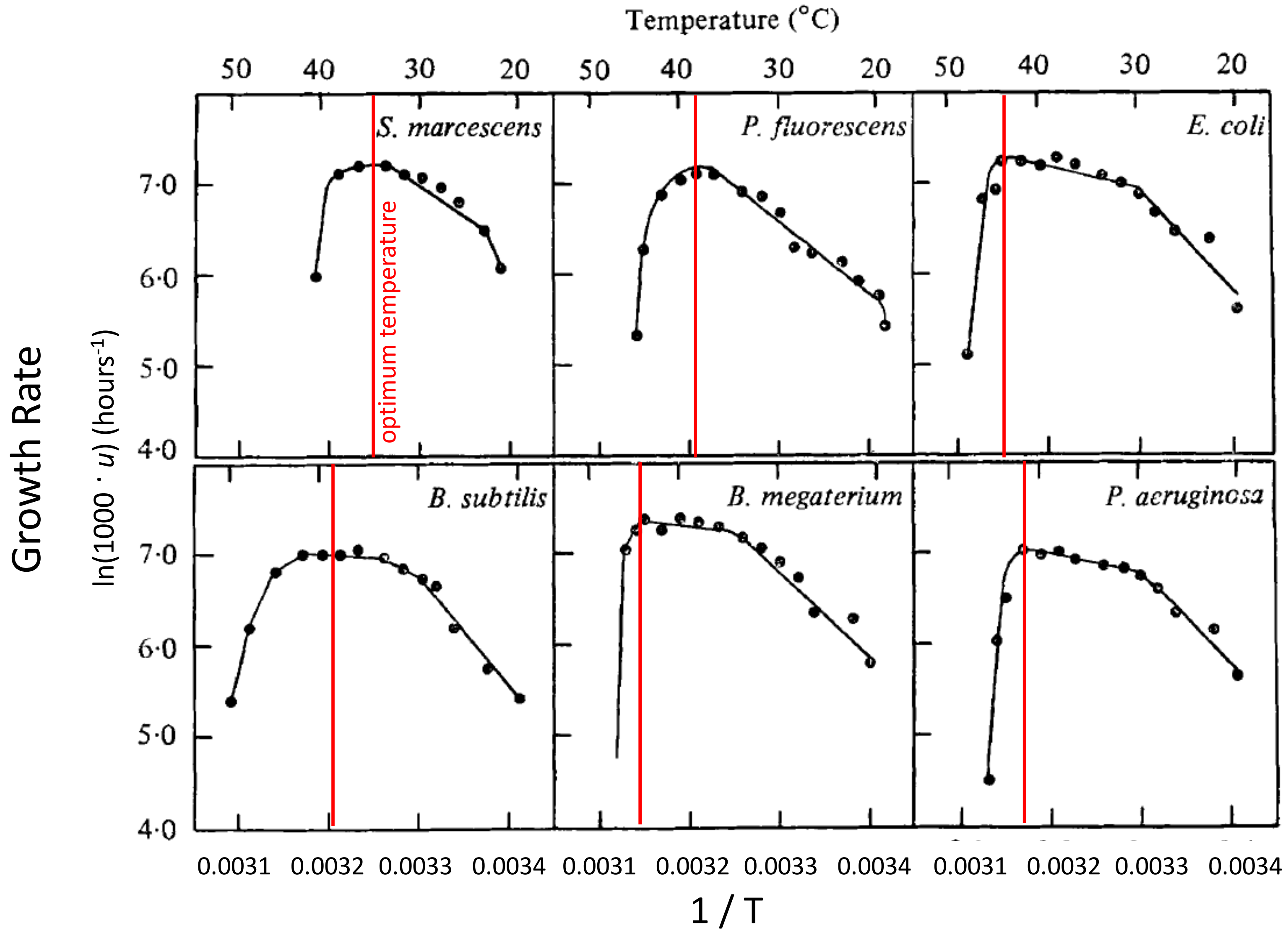
$$\ln(R_{\text{tot}}) = a - b(1/T)$$

with the slope (b) estimating E_a/k_B



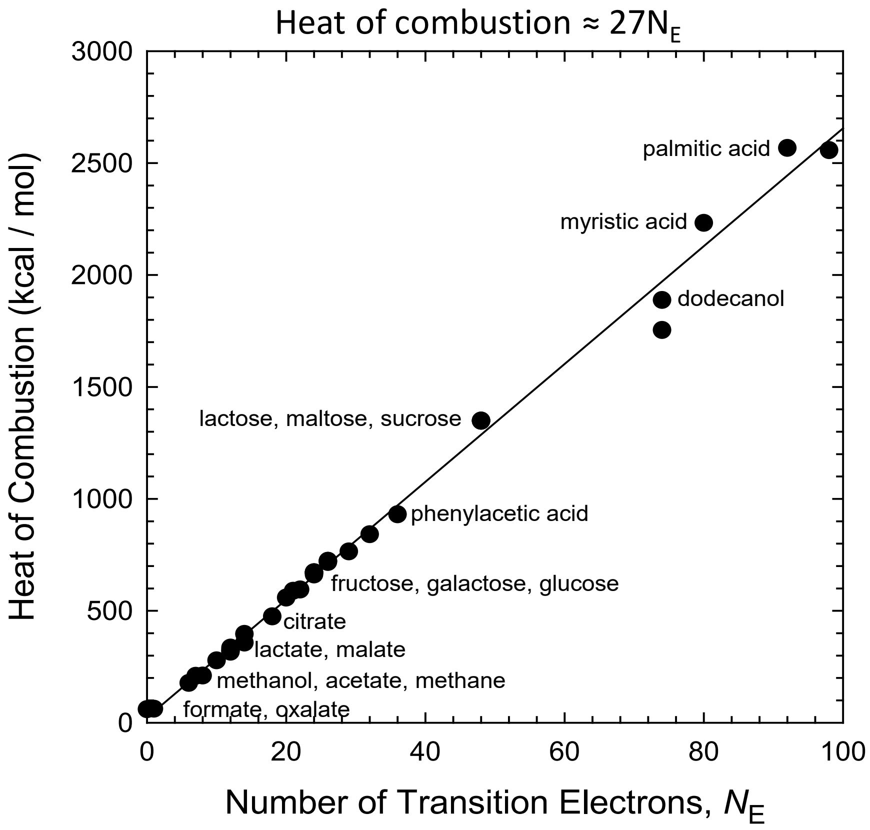
Herendeen et al. (1979, J. Bacteriol.)

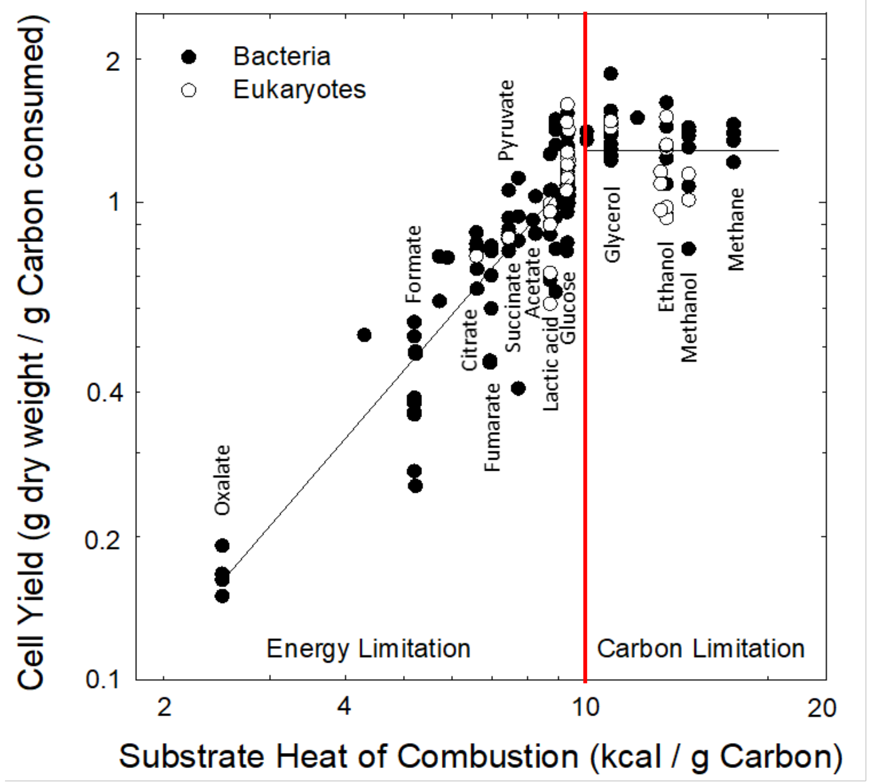
Thermal Response Curves Differ Among Species



Mohr, P. W., and S. Krawiec (1980)

Energy, Carbon Skeletons, and Cell Yield





Carbon Assimilation Efficiency:

- Cells are ~50% C dry weight.
- 0.8 to 1.6 yields imply 40 to 80% maximum carbon assimilation into biomass.
- This is the maximum that natural selection has been able to achieve over a course of 4 billion years of evolution.

Degree of reduction: $N_E = 4N_C + N_H - 2N_O$

Kharasch and Sher (1925) – electrons associated with H are free to move upon combustion; those associated with O are not.

Lynch and Trickovic (2020)

What fraction of resource consumption is diverted to energy production vs. carbon skeletons?

- Assume a high-energy substrate such as glucose (9.3 kcal / g C), with a yield of 1.3 g dry weight / g C consumed.

- Energy requirement for growth = (9.3 kcal / g C) / (1.3 g dry weight / g C consumed) = 7.2 kcal / g dry weight produced.

- Caloric content of an average bacterial cell ≈ 5.3 kcal / g dry weight.

- Food consumption diverted to energy production ≈ 7.2 - 5.3 = 1.9 kcal / g dry weight.
    - ~25% goes to energy production; 75% to carbon skeletons.