

Entropy in Biology

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The second law of thermodynamics makes no distinction between living and non-living things. Indeed, the concepts of thermodynamics constitute the unifying principles of physics, chemistry and biology.

Processes leading to randomness, disorder, chaos or loss of information would appear to be out of place in the world of biology. Living things are characterized by a very high degree of structure and assembly, whether at the level of molecules, genetic information, cells, tissues, organs, organisms or populations of organisms. On the other hand, the second law of thermodynamics implies the principle that the total entropy, which is a measure of disorder, must increase steadily. Even though thermodynamics itself does not describe processes as a function of time, the second law defines a unique direction of time (time's arrow) as the direction in which total entropy increases. Nevertheless, thermodynamics does not exclude local exceptions. Living things are local exceptions.

Every isolated system moves towards a state of maximum entropy. The universe is presumably an isolated system, whose entropy can never decrease. Thus, it is clear that the decrease in entropy that accompanies growth of living structure, must always be accompanied by an increase in entropy in the sustaining physical environment. In his classic book, *What is life?*, the physicist Erwin Schrödinger discussed how life is a state of very low probability because so much energy is needed to create and sustain it. The 'vital force' that maintains life is energy. Living things preserve their low levels of entropy throughout time, because they receive energy from their surroundings in the form of food. They gain their order at the expense of disordering the nutrients they consume. For example, the development of or-

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ganic structure in plants utilizes the energy of sunlight: the energy of light is used to create biomolecules. When the light was produced in the first place, its production was dependent on a large increase in entropy in the Sun!

Life on earth is possible because the temperature is such that it is high enough to permit structure-creating reactions, and yet low enough to ensure that vital biochemical structures are not destroyed by the chaotic agitation from random thermal motion. Life can cease if a relatively small change in temperature occurs in either direction. At lower temperatures metabolism slows down and effectively stops, while at higher temperatures, the specific structural organization in the cell that facilitates metabolic reactions, disappears. It is truly a delicate balance. During the process of ageing, the balance shifts in the direction of anarchy. Death is the thermodynamically favored state: it represents a large increase in entropy as molecular structure yields to chaos. Although human beings expend a lot of energy to avert death, it is a state of too high a probability to be evaded.

Entropy and the Evolution of Life

Progress in nature is nearly always achieved by the development of more complicated biological structures, usually when these structures are better adapted to the environment. This development occurs by accumulation of small random changes in the DNA master plan, not by starting again from scratch. In other words, the mechanism of evolution makes the whole process irreversible. Thus, the analogy between the theory of the evolution of life and the laws of statistical physics and entropy, both of which apply to large populations (of organisms and molecules, respectively), extends to the concept of the irreversibility of time. It is interesting that Darwin developed his ideas on the evolution of life forms at about the same time as his contemporaries, the physicists Clausius, Boltzmann, Maxwell and Gibbs, formulated the laws of statistical physics and entropy. It was Boltzmann who remarked that the nineteenth century would be known as the century of Darwin!



Entropy and Free Energy

According to the second law of thermodynamics, spontaneous chemical reactions, which in biological processes typically occur at constant temperature and pressure, are always accompanied by decreases in free energy. Free energy is energy that is capable of doing work. All reactions proceed towards a state of equilibrium, the state of maximum probability where nothing further happens, and hence, no further decrease in free energy occurs. The free energy lost appears either as heat (enthalpy) or is used to increase the entropy. Thus, the spontaneous chemical reactions, on which life depends, may proceed without release of heat, and may, in fact, proceed with take-up of heat, but only at the cost of expending free energy. For example, the unfolding of a protein consumes a large amount of heat, and as described below, is the ultimate example of a reaction that can be driven by an increase in entropy. The original definition of entropy, which was proposed by Rudolf Clausius in 1864, when applied to biological processes that occur typically at constant temperature, is that the change in entropy is equal to the heat supplied divided by the temperature. Since for a protein unfolding reaction, heat supplied is positive because heat is taken up, the change in entropy is always positive.

Entropy as a Driving Force for Protein Unfolding

One manifestation of the high order and low entropy characteristic of living systems is evident in the intricate and hierarchical, but yet very precise structures of proteins, into which their polypeptide chains comprising amino acid residues fold. It is the structure that determines the function of any protein, whether in catalysis, structural support, locomotion, transport or communication. The functional or native state of a protein has very low entropy because its conformation is highly restricted. On the other hand, the unfolded form can exist in many different conformations: even if each amino acid can adopt only 3 positions, a polypeptide chain of 100 amino acid residues can adopt 3^{100} or 10^{47} different conformations. Since the product of the

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protein unfolding reaction can exist in a larger number of equivalent states, compared with the native state, entropy increases during the unfolding reaction. From the simple formula defined by Ludwig Boltzmann in 1877, the entropy of the unfolded state, which is equal to $k \ln W$ (where k is the Boltzmann constant, and W is the number of accessible states) can be calculated to be $\sim 250 \text{ cal K}^{-1} \text{ mol}^{-1}$. This is more than 10 times higher than the entropy changes of $20 \text{ cal K}^{-1} \text{ mol}^{-1}$, that are usually observed for the reactions of small molecules. Thus, the entropy loss during protein folding plays a much larger role in determining the shape of the free energy reaction landscape than it does in most small molecule reactions.

For a protein to fold, the loss of entropy must be balanced by the gain in enthalpy for the free energy to favor folding. Strong non-covalent forces from hydrogen bonding and other physical interactions compensate for the low entropy of the native state, but just barely. Changes in the entropy of the solvent, water, play an important role in compensating for the loss in conformational entropy.

In the native state, many non-polar amino acid residues are packed into the interior of the protein, and hence, sequestered away from water. In the unfolded form, these residues are exposed to water molecules, which consequently, get arranged around the non-polar residues, into cage-like structures: the normal hydrogen bonding network present in water is reorganized so that the number of hydrogen bonds is nearly preserved. The additional hydrogen bond donors and acceptors of the polar polypeptide backbone of the protein, which get exposed upon unfolding, probably also play a role in restricting the freedom of movement of more water molecules. This ordering of water molecules lowers the entropy of water. When the protein folds, these water molecules are released as the non-polar residues get secluded from water. The resultant regain of entropy by water, is thought to be a dominant force in protein folding, and the effect is commonly referred to as the hydrophobic effect.

According to the original definition by Clausius, the change in entropy is larger at lower temperatures: a fixed amount of heat has a greater disordering effect at a lower temperature than at a higher temperature. The entropy of protein unfolding would therefore be expected to become smaller with increasing temperature. Nevertheless, it is commonly observed that the entropy of unfolding becomes higher with increasing temperature, because the cage-like structures of water molecules melt at higher temperatures, and so fewer water molecules remain restricted in motion. The solvent can therefore have profound effects on biological reactions.

Entropy as a Driving Force for Catalysis by Enzymes

Although life is driven by spontaneous reactions, it is only by the intervention of large, very elaborately structured molecules in cells, usually proteins called enzymes, that the rates of these reactions are brought into biologically relevant time scales. Entropy plays a big role in enzyme catalysis. Reactions in solution are usually slow because of the entropic cost in bringing the reactants or reactant and catalyst together. Two or more molecules associating to form one involves considerable loss of entropy. On the other hand, when an enzyme binds its substrate, the binding energy released is used to compensate for the loss in translational and rotational entropies which occurs on formation of the enzyme-substrate complex, which is a state of very low probability because catalytic groups have to be oriented very precisely, within fractions of an angstrom. This occurs at the expense of an increase in the dissociation constant of the enzyme-substrate complex. Very little entropy loss occurs during the actual chemical reaction steps, because the catalytic groups are already properly oriented on the same enzyme-substrate complex, and hence, their effective concentrations are very high compared to the corresponding bimolecular reactions that occur free in solution. Thus, the ability of enzymes to accelerate chemical reactions by factors as large as 10^{15} , resides in their

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Suggested Reading

- [1] PW Atkins, *The 2nd law. Energy Chaos and Form*, Scientific American Books, 1994.
- [2] R A Abeles, P A Frey and P Jencks, *Biochemistry*, Jones and Bartlett Publishers, 1992.
- [3] F Jacob, *The Logic of Life*, Random House, Inc, 1982.
- [4] M Eigen and R Winkler, *The Laws of the Game*, Princeton University Press, 1993.
- [5] T E Fischer, A F Oberhauser, M Carrion-Vazquez, P E Marszalek and J M Fernandez, The study of protein mechanics with the atomic force microscope, *Trends in Biochemical Sciences*, 24, pp.379-384, 1999.
- [6] R D Astumian, Making molecules into motors, *Scientific American*, 285, pp.57-64, 2001.

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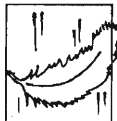
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ability to reduce the amount of entropy that must be lost for the reaction to occur.

Entropy and Single Molecules in Biology

One of the most exciting developments in biology today is the application of physical methods to study the behavior of single protein or DNA molecules. For instance, the use of optical tweezers and atomic force microscopes now makes it possible to take hold of a single multi-domain protein molecule and mechanically stretch it, thereby unfolding it. A fully stretched protein molecule has near zero entropy. When it is freed from mechanical restraint, it first adopts a random coil unfolded form of high entropy, and then folds to the native state which again has near-zero entropy. Measurement of the entropic restoring force in a single stretched protein molecule allows direct determination of the mechanical force and energy that can unfold a single protein domain. The methods of single molecule biology are now being applied to many diverse mechanical systems in biology, including molecular motors.

Molecular motors carry out the basic functions that keep cells living: these protein molecules build or destroy other proteins, transport materials, pump ions, or propel the cell. They do their jobs by converting chemical energy into mechanical energy with near 100% efficiency. The directed motion of molecular motors, overcoming entropy, is crucial for processing information in biology. It appears that biological nanomotors might be able to perform their tasks because they use the energy from biased Brownian motion to convert the molecular disorder around them into order. Nothing escapes the long arm of the second law.



Infinites and indivisibles transcend our finite understanding, the former on account of their magnitude, the latter because of their smallness; Imagine what they are when combined.

Two New Sciences, 1638