

possible to cause nucleic acid molecules to evolve in the test tube. With the discovery of catalytic RNA, and advances in nucleic acid amplification techniques such as the polymerase chain reaction, it became possible to construct laboratory systems that allow the Darwinian evolution of functional RNA (and later DNA) molecules. This is not evolution based on natural selection, but rather directed evolution based on selection constraints imposed by the experimenter. We've been playing these *in vitro* evolution games in my own laboratory for the past 10 years.

The counterforce, it turns out, is not much to look at — typically, 20 microliters of a clear, colorless solution. But in those solutions the Titans are rumbling. We can begin in the lab on a Monday with a population of 10^{14} random-sequence nucleic acid molecules and by Friday witness the emergence of order in the form of macromolecules of a particular sequence that perform a specific catalytic task. Over the week, the evolving system has expended more than 10^{17} energy-rich nucleoside triphosphates, but has created an island of order in a universe that is forever tumbling toward a state of maximum entropy.

References

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The editors of *Current Biology* have invited a number of biologists to reveal the work that has influenced them most profoundly in their careers. These brief essays are published in the *Turning points* series. If you have any comments, or ideas arising from this series, we shall be happy to consider them.

Quick guide

Hydrogen bonding

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What is it? A hydrogen bond is an interaction in which a hydrogen atom bridges two electronegative atoms (in biological systems, usually nitrogen or oxygen).

Why is it important in biological systems? Hydrogen bonds hold the two strands of a DNA helix together, and in proteins they hold α -helices and β -sheets together. They are perfect for the job — weaker than covalent bonds but strong enough to provide specificity and directionality in, say, DNA replication.

Where would we be without it? Unstuck.

So, what defines a hydrogen bond?

Classically, a hydrogen bond is said to exist in an A-H...B system (where A and B are the 'end atoms' of the bond), when the distance between H and B is significantly less than the sum of the van der Waals radii of H and B. Also, the angle A-H...B should be near 180° . Typically, any distance between H and B of less than 3.5 \AA , with a deviation of the angle from linearity of less than 30° is considered to be a hydrogen bond. Well, you did ask.

How does it work? Put simply, a hydrogen bond involves an attraction of an electron donor atom ($B^{\delta-}$) for a proton donor group ($A^{\delta-}-H^{\delta+}$). As the hydrogen bond gets stronger, there is more electron delocalization and covalent character in the hydrogen bond, until the hydrogen is fully shared between the end atoms, A and B. In strong, short hydrogen bonds, the hydrogen atom is located midway between the end atoms and the distance between these end atoms is $2.3-2.7 \text{ \AA}$. Such bonds are often called 'low-barrier' hydrogen bonds.

How do I know if it's a hydrogen bond?

Neutron diffraction crystallography is the most definitive way to detect a hydrogen bond but low-temperature x-ray crystallography can also be used. Hydrogen atoms involved in hydrogen bonding are shifted downfield in NMR data and red-shifted in vibrational spectroscopy data, so these are the best ways to determine whether a molecule is hydrogen bonded in solution.

How strong is it? Hydrogen bond strengths can vary from 5 kJ/mole to hundreds of kJ/mole, depending on the molecules and whether they are in a gas or a solution. For example, a guanine-cytosine hydrogen bond has a strength of about 80-100 kJ/mole in the gas phase and less than 20 kJ/mole in aqueous solution.

How much is a hydrogen bond worth in a protein? The answer to this question is much debated and, so far, unresolved. Site-specific mutations that convert hydrogen-bonding amino acids to amino acids that don't hydrogen bond often have little effect on the free energy of protein stability or binding; however, this could be because any effects are counterbalanced by changes in the residue's hydrogen bonding with the aqueous solvent.

Why is there so much fuss about low-barrier hydrogen bonds?

Many people think that low-barrier hydrogen bonds are important in enzyme catalysis, but these arguments are often based on hydrogen bond strengths in the gas phase, which can be misleading. No one disputes the existence of low-barrier hydrogen bonds, which can be detected using NMR, but it's not clear whether they have any real energetic consequences for catalysis.

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