Everyone who has taken a chemistry course has been exposed to the concept of a hydrogen bond (HB). When a H atom is covalently attached to another atom A, typically an electronegative one such as O, N or F, the A-H bond is polarized with the H acquiring a partial positive charge. The latter can then attract an atom (D) from another molecule that contains a partial negative charge, with O, N and F being prominent examples. This AH-D HB amounts to something in the order of 3–10 kcal/mol, at least an order of magnitude weaker than a covalent bond, but nonetheless with enormous implications. These HBs are of particular importance in water, where they control many of its properties, as well as the ability of molecules to dissolve in this ubiquitous solvent. The HBs between nucleic acid bases in DNA are a linchpin of the fidelity of the genetic code and its transmission. This phenomenon also plays a major role in the structure adopted by proteins, whether α-helix, β-sheet or other geometries. There are a host of catalytic processes that employ HBs as a crucial component in their mechanism.

So, what do we really know about these HBs? The strength of HBs is highly variable. HBs strive for linearity in their AH-D arrangement, but of course there are usually other constraints within the system as a whole that pull the HB away from this optimal arrangement, albeit at some energetic cost. Along the same line, each HB has a preferred length, e.g., 2.9 Å (angstroms), for the water dimer, but again macromolecular constraints can stretch these intermolecular distances. A more electronegative A proton donor atom is advantageous as is a more basic proton acceptor D. The strength is amplified if the donor molecule bears a positive charge, and/or the acceptor is an anion. In terms of the origin of their strength, the Coulombic attraction mentioned above is only part of the story. The proton acceptor typically transfers a certain amount of charge into the σ*(AH) antibonding orbital of the proton donor, which weakens this bond, and results in the well-known red shift of the ν(AH) stretching frequency. Some electron density drains from the bridging proton which is responsible for the downfield shift of its nuclear magnetic resonance (NMR) signal, another diagnostic of a HB.

What has been learned about HBs in the last few years that is new, perhaps not usually covered in undergraduate courses? The list of elements that can participate in HBs has expanded impressively from the original set of O, N and F. The current list of active participants includes all of the halogen atoms, and most of the chalcogens (S, Se, etc.) and pnictogens (P, As and so on). There is growing evidence that metal atoms can involve themselves in HBs, both as proton donors and acceptors. The earlier inceptions of HBs had the proton acceptor atom interacting with the bridging proton through one of its lone electron pairs. But again, later work revealed other sources of electron density, such as the π-clouds of alkenes or aromatic systems. Even the σ-orbital of a molecule as simple as H₂, cycloalkane or Be₂ can act as a proton acceptor. Another proton acceptor could be the H atom of a different molecule. This variant of a HB, commonly dubbed a dihydrogen bond AH···HM, requires one of the H atoms to have a slight positive charge, as is normally the case, but the other H must be oppositely charged, as would be the situation if bonded to a metal atom M. Not all HBs involve closed-shell systems, as radicals can participate as well. This broadening of the original conception of the HB has motivated a recent redefinition by the International Union of Pure and Applied Chemistry (IUPAC) that eschews its classification via participating atoms, in favour of a list of properties, e.g., geometry, vibrational and NMR spectra, and electron density perturbations.

Other than the venerable ideas concerning how HBs contribute to biomolecular structure, what are some of the newer ideas that have emerged from recent studies of HBs?
Proton Transfer

First, the transfer of a proton from one group to another within a HB is a common part of the mechanism of a wide variety of enzymes. How can a protein facilitate this transfer? Quantum calculations have shown that the energy barrier to proton transfer ($p_T$) is highly sensitive to the length of the HB. A stretch of the distance between the proton donor and acceptor groups by only 0.2 Å can raise the barrier quite a bit. This rise has profound implications for the rate of transfer which in turn decays exponentially with any barrier increase. Of course, the transfer of a very light proton can occur via quantum mechanical tunneling, rather than a classical ride over the top of the barrier, but this process is also very sensitive to the height of the barrier. The overall conclusion is that the HB can be thought of as a spark plug in some ways. With a short gap between the two electrodes, the spark (proton) can easily jump across, but if the gap is widened by even a small HB stretch, the gap will no longer permit the proton transfer, as cartooned in Figure 1.

Another important aspect of HBs is their angular characteristics. While a HB prefers a perfectly linear $AH \cdot \cdot \cdot D$ alignment in its perfect world, the interior of a protein is hardly a perfect world. There are myriad geometrical factors that determine a protein's structure, which is a compromise between all of these issues. So, in fact, protein HBs are far more often bent than they are linear, in the same way that few HBs within proteins can adopt their most favourable bond length. One consequence of any such angular distortion is a rise in $p_T$ barrier height, which acts in the same way as a bond stretch to slow down and even prevent the proton transfer.

However, there is a second, more unexpected and perhaps useful consequence. The angular characteristics of a HB can actually push the proton from one group to another. Consider for example, a pair of protein residues, A and D, involved in a HB. If A is more basic than D, the proton would tend to favour A, and indeed, the $AH^+ \cdot \cdot \cdot D$ structure would be favoured, if the HB is linear. But there are certain angular misalignments that would shift the equilibrium position of the proton toward D. In such a bent HB, it would be the $A \cdot \cdot \cdot H^+D$ configuration that would be more stable. (It should be stressed that this apparent paradox is not magical but is easily explained on the basis of very simple Coulombic arguments.) A protein could take advantage of this principle. While the resting or starting state of an enzyme could have the proton on the more basic A, when the appropriate step of the mechanism has been reached and a proton transfer is called for, it would take only a slight conformational change to adjust the HB geometry, and push the proton across the HB to group D. This principle is illustrated in Figure 2 for the HB in bacteriorhodopsin connecting the N atoms of a Schiff base and a Lysine residue.

CH···O H-bonds

As mentioned earlier, the original inception of a HB that is limited to F, O or N atoms has undergone a radical broadening to also include an array of other atoms. Well, what is more common within a molecule, particularly a protein, than the CH group that occurs along the protein...
backbone, as well as in numerous amino acid side chains? Due to the similar electronegativities of C and H, the conventional wisdom had been that the H atom would not be able to acquire the partial positive charge needed to engage in a HB. Well, as often happens in science, the conventional wisdom was wrong. It is true that simple alkanes are unable to engage in HBs. But a number of even minor alterations can change things. It is known that a change in hybridization from sp³ in alkanes results in a more electronegative C. It is for this reason that HCCCH, with its sp hybridization is able to act as a proton donor in HBs. An even more important factor emerges from substitution. An electron-withdrawing substituent on the C, even sp³ C, acts to pull electronic charge away from the H on this C atom, imbuing it with a partial positive charge and thus able to participate in a HB. CF₃H is thus a strong proton donor for this very reason.

The CαH of proteins is a biological case in point. This C is bonded on either side to a peptide group, which draws charge away from the H. There is thus evidence that the CαH is involved in HBs within proteins. It is true that CH-O HBs will tend to be weaker than their more standard NH-O counterparts but their effects can hardly be ignored. For example, the prevalence of the β-sheet in proteins is generally explained by the NH-O HBs between one strand and its neighbour. But the CαH groups are also positioned so that an interstrand CH-O HB is possible. And indeed, calculations have suggested that the latter CH-O HBs are part and parcel of the stabilizing interstrand attraction, only slightly weaker than, or on a par with, the NH-O HBs, both of which are shown in Figure 3.

Other work has suggested that these CH-O HBs can play important roles in enzyme functioning, beyond a simple structural effect. In a broader context, the sheer number of CH groups within proteins makes them a force to be reckoned with. Even if each one is fairly weak, when multiplied by their number their sum contribution to the overall stability of the native conformation can hardly be ignored. The recent establishment of the idea of a CH-O HB has led researchers to re-examine many of the older protein structures so as to identify their presence, and to consider inclusion of this force into empirical potentials used to refine protein structure.

As an interesting historical aside, it turned out that many of these CH-O HBs have a quirk. Rather than shift the A-H IR stretching frequency to the red as was typical of HBs, some of them shifted in the opposite direction. Although all of the other features of these interactions fit the HB fingerprint nicely, this oddity led some early researchers to deny their nature as true HBs. To this day, these blue-shifting HBs are sometimes referred to as unconventional, non-traditional or even anti-HBs.

 Extensions of H-bonds

By its very definition, a HB places a H atom between another pair of atoms. But can we replace the H itself and still have something that looks like a HB? What would happen if this replacement atom were electronegative, like a halogen. Of course, the electron-withdrawing power of the halogen atom X would preclude this atom acquiring a partial positive charge, so one would not have the A-X+ character that is part of the HB package. However, it must be considered that the X atom does not need to have a partial positive charge that fully envelops it. Wouldn’t it be sufficient if there were a region of positive charge facing the base group D, even if the rest of the region surrounding the X atom had a negative charge?

It turns out that this is indeed the case. The electrostatic potential that surrounds the A-X bond can be described as a positive region along the extension of the A-X bond, surrounded by a belt of negative charge as a sort of equator to the pole of positive charge, as illustrated in Figure 4. The
total negative charge of this belt overwhelms the positive polar region, so the X atom is in fact partially negatively charged, as expected for an electron-withdrawing halogen atom. However, the positively charged pole, commonly referred to as a ‘σ-hole’, is fully capable of attracting a base D, in just the same way as a proton within a HB. The ensuing non-covalent bond has been dubbed a halogen bond (XB) and has been known for some time but is enjoying a rejuvenation of study in recent years. This impressive body of work has shown that a XB is almost a precise parallel of a HB, with common underlying attractive forces, sensitivity to deformation and with similar total strength.

If a halogen atom can substitute for H, why not others as well? Indeed, a wide range of electronegative atoms are capable of forming the same sort of non-covalent bonds. Depending upon the periodic family in which they lie, these attractions are typically called chalcogen (S, Se, etc.), pnictogen (P, As) and tetrel (Si, Ge) bonds. And like their halogen bond cousin, these bonds are also capable of strengths that rival the HB. (The reader may note that these lists exclude the elements from the periodic table first row [F, O, N, C] for the simple reason that these small atoms tend to resist such activity but can be induced to form these bonds under certain circumstances.)

As this variety of σ-hole non-covalent bonds is creeping into the consciousness of practising chemists and biochemists, they are making their presence felt in a variety of systems. The common occurrence of halogen atoms in pharmaceutical agents makes the XB an important player in their biological activity. Even the tetrel bond involving the methyl group, weak as it is for its usage of the C atom, appears to be quite common in protein structures, and seems to play a role in the activity of certain enzymes.

So, even after a century of inquiry, the HB is still offering researchers new insights, applications and spin-offs. It would be premature, and even arrogant, for us to close the book on study of this interaction and declare it fully understood.

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**Further reading**