Six Lectures on the Nature of the Hydrogen Bond
Lecture 5
H-Bond Patterns in Nature:
A Gallery of Functional H-Bonds

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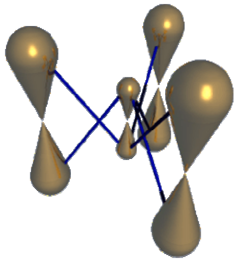
The topics of the present lecture have been previously presented to other meetings and, in particular, to:



I N D A B A I V

Workshop on: **Patterns in Nature**

Skukuza, Kruger National Park, South Africa

17 - 22 August 2003

Hydrogen Bond Patterns in Nature

Paola Gilli, Loretta Pretto and Gastone Gilli

ECM22

22nd European Crystallographic Meeting

26-31 August 2004 - Budapest, Hungary

Smart H-Bonds in the Design of Crystals, Molecular Devices, and Functional Materials

Paola Gilli, Loretta Pretto and Gastone Gilli

Syngenta Crop Protection, 4332 Stein, CH, 28 October 2010

H-Bond Patterns in Nature.

A Gallery of Functional H-Bonds

Gastone Gilli

CUSO Summer School 2012 on Hydrogen Bonding

20-24 August 2012 Villars sur Ollon, Switzerland

Six Lectures on the Nature of the Hydrogen Bond

Gastone Gilli

Traditional H-bond definitions

Definition 1. The Hydrogen Bond (H-bond; Pauling, 1931)

The *Weak H-Bond* is mostly an *electrostatic interaction*

$$R\text{–}D^{\delta-}\text{–}H^{\delta+}\cdot\cdot\cdot :A^{\delta-}\text{–}R'$$

while the *Strong H-Bond* is mostly a *covalent three-center-four-electron bond*

$$R\text{–}D\cdot\text{–}\cdot H\cdot\cdot\cdot :A\text{–}R' \leftrightarrow R\text{–}D:\cdot\cdot\cdot \cdot H\cdot\text{–}\cdot A\text{–}R'$$

between a *proton donor* D-H {where D is a strongly electronegative atom such as F, O, N, C, S, Cl, Br, I}

and a *proton acceptor* or *electron-pair carrier* :A {a second electronegative atom or the π-bond of a multiple bond}

Definition 2. The Hydrogen Bridge (H-bridge; Hugging, 1936)

The H-bridge is a proton-sharing interaction

$$R\text{-}D^{-}:\cdot\cdot\cdot H^{+}\cdot\cdot\cdot :A\text{-}R'$$

in which the central proton H^+ ***shares two electron pairs coming from two adjacent electronegative atoms***

How the H-bond really works: The dual H-bond

R–D⁻: · · · H⁺ · · · :A–R'

Following the H-bridge concept, the H-bond is not really *a bond*
but consists of *two bonds* made by the central proton with the two adjacent acceptors,
so that all H-bond properties (in particular, its energy E_{HB}) must depend on two variables
able to quantify the relative strengths of the two bonds formed.

These two variables can be taken as two proton affinities, pa(D⁻) and pa(:A)
or, better, as their linear combinations Sum and Difference,
which only have a precise physical meaning. Hence,

$$E_{HB} = f\{[\text{Sum}];[\text{Difference}]\} = f\{[\Sigma pa]; [\Delta pa]\},$$

where

Sum= Σpa= [pa(D⁻) + pa(:A)] ≈ [χ(D)+ χ(A)]/2 = Mean electronegativity of D and A

Difference= [Δpa= pa(D-)– pa(:A)] ≈ $\Delta pK_a = pK_{AH}(D–H) – pK_{BH^+}(A–H^+)$ =
Thermodynamic acidity difference between D–H and A–H⁺.

Both Sum and Difference exert a profound influence on the value of E_{HB}, as
it will be shown in the next slide.

Strength rules from the dual H-bond model

The Sum Rule: ***The Electronegativity Rule***

Let's call $E_{HB,MAX}$ the maximum H-bond energy achievable when $\Delta pa = \Delta pK_a = 0$,
Gas-phase data show that $E_{HB,MAX}$ depends on D/A electronegativities according to the series:

FHF > OHO > NHN > ClHCl > BrHBr > NHO > SHS
42 > 27-32 > 29-31 > 24 > 19 > 15-20 > 13
kcal mol^{-1}

The Difference Rule: ***The pK_a Equalization Principle***

$\Delta pK_a >> 0$: D-H····A, weak & neutral
$\Delta pK_a \approx 0$: D···H···A, strong & centered; $E_{HB} \rightarrow E_{HB,MAX}$
$\Delta pK_a << 0$: $^-$D····H-A$^+$, weak & charged

The Chemical Rule: ***The H-Bond Chemical Leitmotivs***

Since all strong H-bonds need that $\Delta pa = \Delta pK_a = 0$, really strong bonds occur only in chemical situations of full p*K*a matching.
These chemical situations are called: "The chemical leitmotifs"

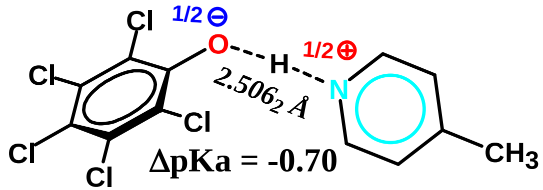
Problem-Solving Procedure: ***How to predict the H-Bond Strengths***

1. **The simple concept of Chemical Leitmotif may help to single out strong H-bonds irrespective of the complexity of the molecular or macromolecular system investigated.**
2. **The Sum and Difference Rules above may help us to predict H-bond energies knowing only the chemical constitution (chemical formula) of the interacting bodies.**

A library of strong H-bond types: The chemical leitmotifs

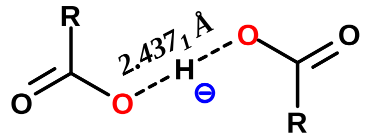
CHARGE–ASSISTED H-BONDs

CL # 1: (±)CAHB ⇒ S, VS
Double Charge-Assisted HB
Direct Acid-Base PA/pK_a Matching



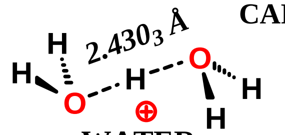
PENTACHLOROPHENOL - *p*-TOLUIDINE

CL # 2: (–)CAHB ⇒ S, VS
Negative Charge-Assisted HB
Acid-Acid PA/pK_a Matching by Proton Loss



CARBOXYLIC ACID - CARBOXYLATE

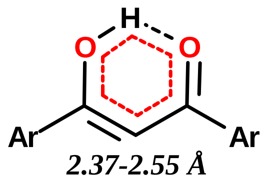
CL # 3: (+)CAHB ⇒ S, VS
Positive Charge-Assisted HB
Base-Base PA/pK_a Matching by Proton Gain



WATER - HYDRONIUM

S= Strong, VS= Very Strong, M= Medium Strong, W= Weak H-Bond

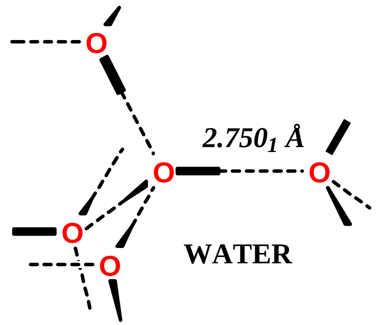
σ/π–BOND COOPERATIVE H-BONDs



DIBENZOYLMETHANE ENOLS

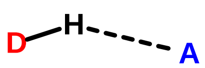
CL # 4: RAHB ⇒ S, VS
Resonance-Assisted HB or π-Cooperative HB
PA/pK_a Matching by π-Conjugated-Bond Polarization

CL # 5: PAHB ⇒ M, W
Polarization-Assisted HB or σ-Cooperative HB
(Partial) PA/pK_a Matching by σ-Bond Polarization

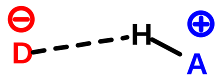


WATER

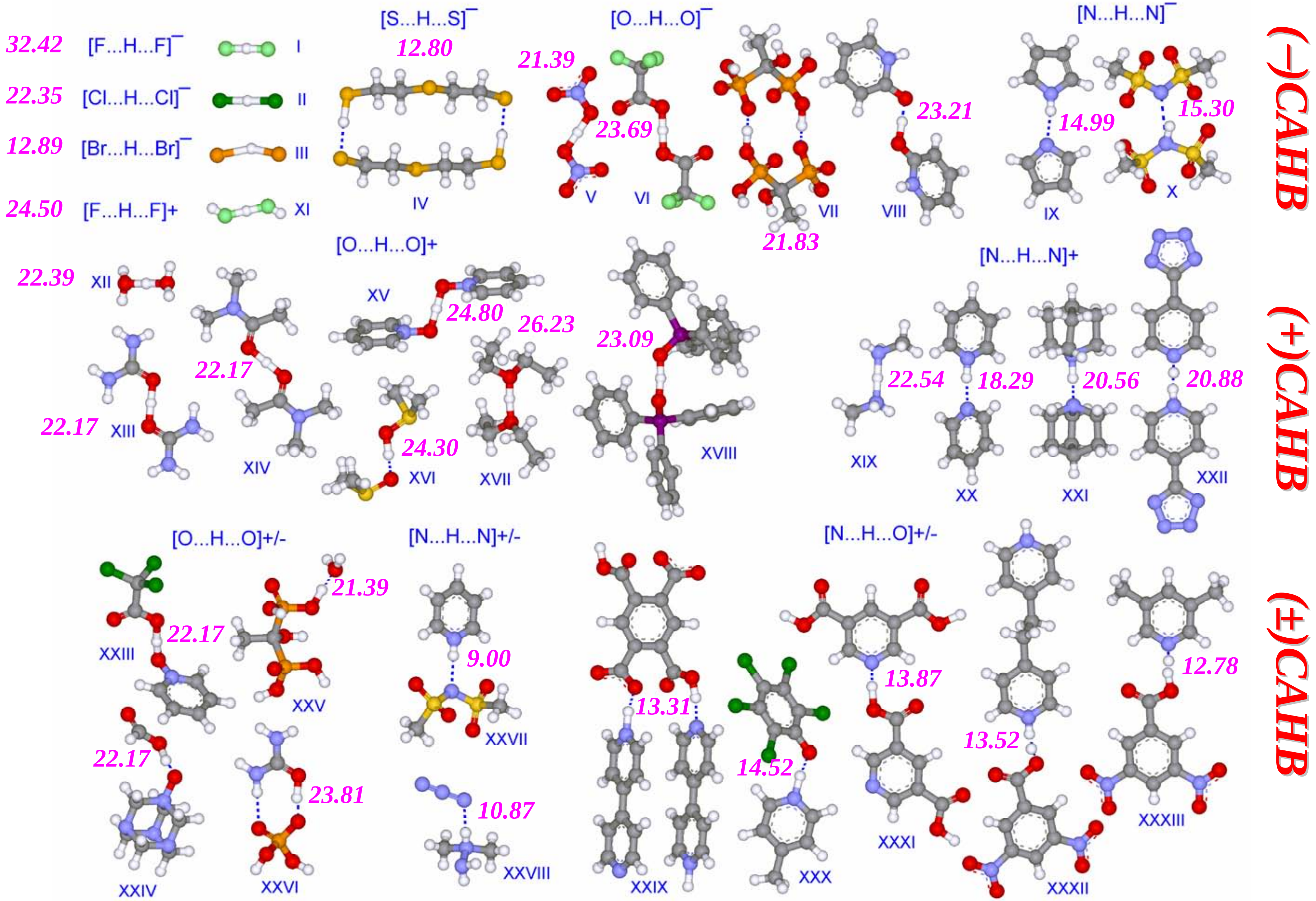
NEITHER CHARGE– NOR RESONANCE/POLARIZATION–ASSISTED H-BONDs



CL # 6: OHB ⇒ W
Ordinary HB
No PA/pK_a Matching



A gallery of most famous strong H-bonds



P. Gilli et al., *Acc. Chem. Res.* (2009); E_{HB} values (kcal mol^{-1}) calculated by the exponential equation

H-bond strength thermodynamics

H-bond strengths are normally given as energies (E_{HB}) or, better, enthalpies ($\Delta H°$). However, what H-bonds do in the chemical environment does not depend on energies or enthalpies but rather on standard free enthalpies, $\Delta G° = \Delta H° - T\Delta S°$, which, in turn, determine their association (K_a) or dissociation (K_d) constants

$$\Delta G° = \Delta H° - T\Delta S° = -RT \ln K_a = RT \ln K_d.$$

But, how are $\Delta G°$ and $\Delta H°$ related? They are related by

The H-Bond $\Delta G°/\Delta H°$ Rule

"In any H-bond, $\Delta G°$ is grater (that is, less negative) than its corresponding $\Delta H°$ because the bond is an association process which takes place with loss of degrees of freedom and then with a systematic decrease of the standard entropy, $\Delta S°$, which is the greater the more tightly the two interacting molecules are held together"

The E–E Compensation Rule

Accordingly, enthalpies and entropies are not independent but related by an *enthalpy-entropy compensation*, $\Delta H° = \beta \Delta S°$, relationship with compensation temperature β (what is called an *extrathermodynamic* relationship)

H-bond strength thermodynamics (cont.)

E–E Compensation. **Values of β come from experimental data collected in different media:**
gas phase, non-polar solvents, molecular crystals (and, most likely, water solutions).

Preliminary data show that the enthalpy loss caused by E-E compensation is

$$\Delta G^\circ = \Delta H^\circ - \beta \Delta S^\circ \approx 0.75\ \Delta H^\circ$$ **in the gas phase,**

but that it is much greater in condensed phases, becoming

$$\Delta G^\circ = \Delta H^\circ - \beta \Delta S^\circ \approx 0.28\ \Delta H^\circ$$ **in polar and non-polar solvents and in molecular crystals.**

This last equation leads to the following table of approximate values :

Data in CCl_4 or similar non-polar organic solvents	$-\Delta H^\circ$ kcal/mol	$-\Delta G^\circ$ kcal/mol	$\log K_a$ M^{-1}	K_a M^{-1}
	1	0.28	0.21	1.6
	5	1.42	1.04	11.
	10	2.81	2.08	120.
	20	5.69	4.17	14,800.
	30	8.72	6.40	2,512,000.

Thermodynamic conclusions (in condensed phases)

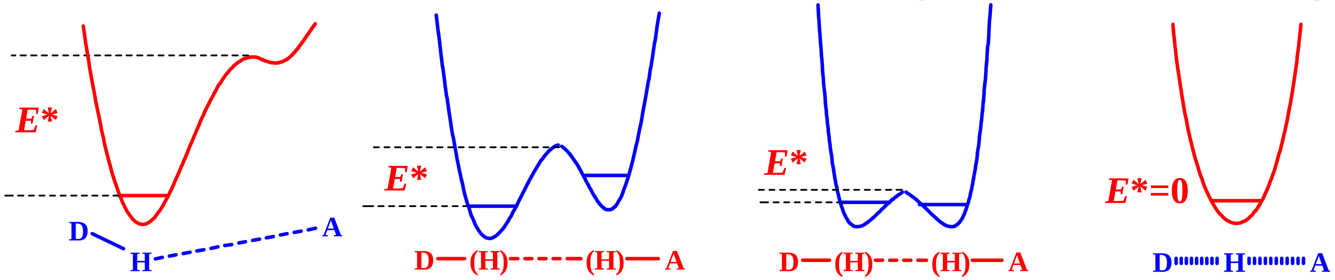
H-bonds can span a total K_a range as wide as 0.2—$2.5 \cdot 10^6$ M^{-1}, which means an increase of eight orders of magnitude in total and of 11 times every 3 kcal mol^{-1} of ΔH°.

This stresses the enormous importance of strong H-bonds which will inevitably dominate the energy landscape of any H-bond-driven process of supramolecular association occurring in crystals or in solution.

The importance of strong H-bonds in chemical kinetics

The strength of the H-bond is an important factor also in chemical kinetics because the <u>H-bond strength</u>, the <u>shape of the PT profile</u>, and the <u>height of the PT barrier</u> are strictly related, <u>PT barriers</u> being large in *weak aSW H*-bonds, rapidly decreasing in strong DW H-bonds, and finally disappearing in *very strong* and proton-centred sSW H-bonds.

Weak HB | **Medium HB** | **Strong HB** | **Very strong HB**



aSW-HB *Asymmetric Single-Well High-Barrier*

aDW-MB *Asymmetric Double-Well Medium-Barrier*

sDW-LB *Symmetric Double-Well Low-Barrier*

sSW-NB *Symmetric Single-Well No-Barrier*

As a consequence,
the H-bond enthalpy $\Delta H°$ basically determines the eight of the PT barrier (E*) and this, in turn, the PT rate constant (k_{PT}) through the Arrhenius equation.

This fact is of outstanding importance in <u>chemical kinetics</u> because proton transfer is an important step in many chemical and biochemical reactions and, in particular, in general acid-base catalysis where protonation of the substrate is mostly assured by ancillary H-bond formation.

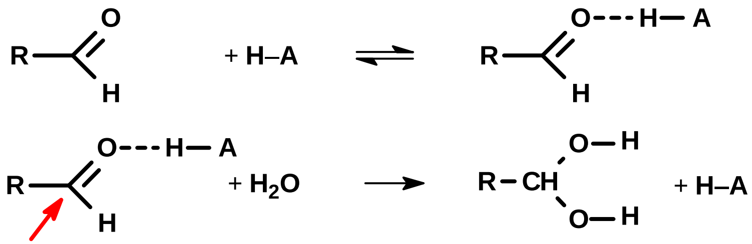
The importance of strong H-bonds in chemical kinetics (cont.)

Two simple examples of H-bond-assisted general acid-base catalysis

[From: H. Maskill, *The physical basis of organic chemistry*, Oxford Univ Press, Oxford, 1985]

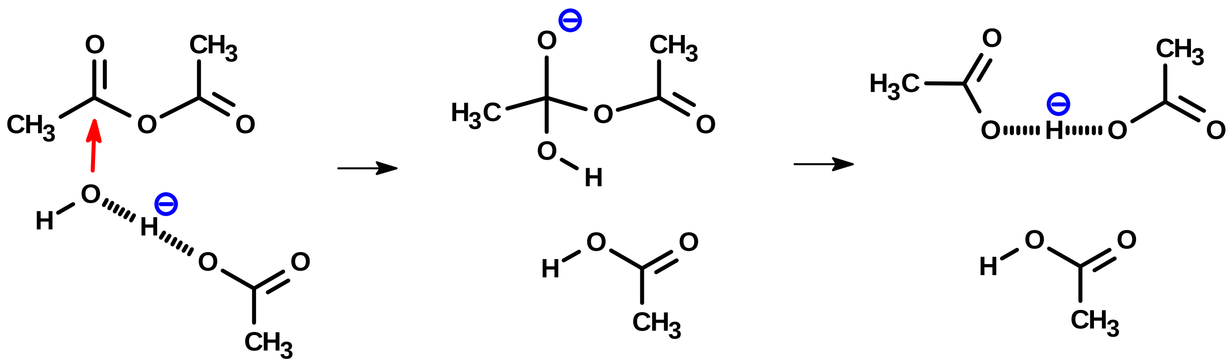
A. Hydration of simple aldehydes in aqueous acidic solutions

The general acid catalysis mechanism involves the formation of an A–H···O bond between the general acid A–H and the substrate, which enhances the substrate electrophilicity in respect to the nucleopilic attack of water.



B. Hydrolysis of acetic anhydride in acetate-buffered solutions

The general base catalysis mechanism involves the formation of a strong [O···H···O]⁻ bond between the general base AcO⁻ and a molecule water, which enhances the nucleophilicity of water during its nucleophilic attack to the substrate.



The concept of 'functional H-bond'

It seems impossible that Nature, in its four billions of years of evolution on Earth, has not taken full advantage of the particularities of weak, strong, and very strong H-bonds.

To check this hypothesis, we have recently screened a large number of molecular and biomolecular processes where H-bonds were supposed to play a role in their mechanisms of action, finding that:

♣ strong H-bonds are quite uncommon but, when occurring, appear to be inevitably involved in some particular aspect of the working mechanism of the system;

♣ the range of phenomena these strong H-bonds are implied in is surprisingly wide, including: organic reactivity, prototropic tautomerism, functional materials, protein and DNA structure (RAHBs); enzymatic catalysis (CAHBs); proton conduction in water and transmembrane channels (PAHBs and CAHBs).

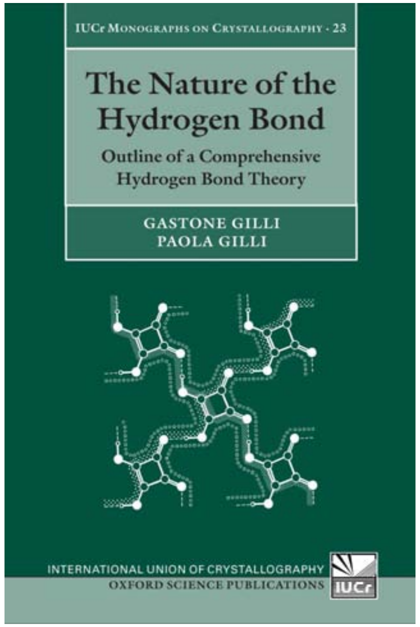
Just to give them a name, we have called this strong H-bonds:

♠ *functional H-bonds*, because they appear to exert a functional role in so many different systems,

♠ though *smart H-bonds* could also be appropriate, because many of them surprisingly seem to behave in an intelligent way.

H-Bond Patterns in Nature
&&&
A Gallery of Functional H-Bonds

From: G. Gilli, P. Gilli - *The Nature of the Hydrogen Bond. Outline of a Comprehensive Hydrogen Bond Theory*, Oxford University Press, Oxford, 2009; Chapter 8.



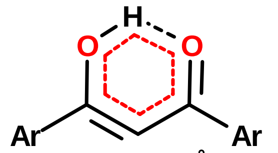
1. RAHB-driven phenomena

1.0 The Resonance-Assisted H-Bond (RAHB)

CL # 4: RAHB $\Rightarrow$ S, VS

Resonance-Assisted HB or π-Cooperative HB

PA/pK_a Matching by π-Conjugated-Bond Polarization

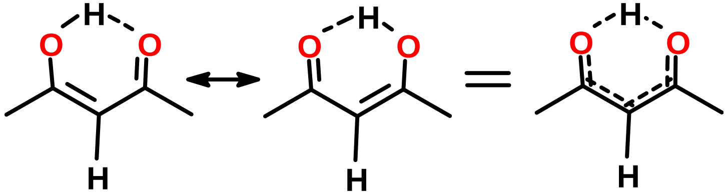


2.37-2.55 Å

DIBENZOYLMETHANE ENOLS

RAHB is a <u>synergistic reinforcement between</u> <u>H-bond strengthening</u> and <u>π-delocalization enhancement</u> occurring when the donor (acid) and the acceptor (base) <u>are connected by a short π-conjugated fragment</u> such as ···O=C–OH··· or ···O=C–C=C–OH···

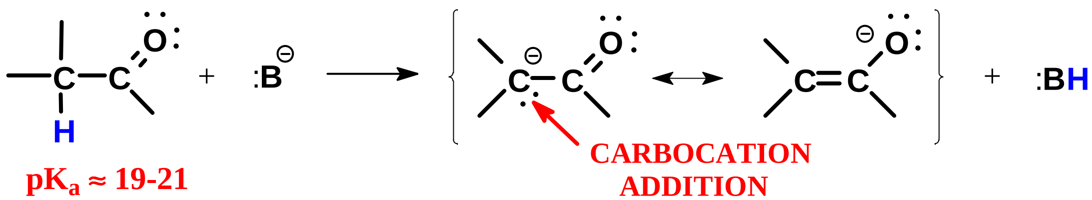
RAHB is unique in chemistry. Its strength is always due to the donor-acceptor pK_a matching but this matching, instead of being caused by the donor/acceptor intrinsic properties, is generated by the strengthening of the H-bond itself through the increased π-resonance within the heteroconjugated fragment:



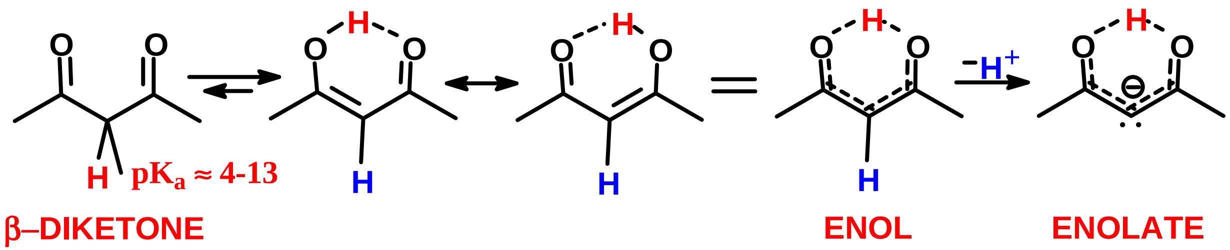
EXAMPLE: β–DIKETONE ENOL

1. RAHB-driven prototropic tautomerism
1.1 Activation of the carbon in α to a carbonyl

Electrophilic addition to the α-carbon involves an enolate ion intermediate and, unless catalyzed by strong bases, proceeds with difficulty due to the low C–H acidity (pKa ≈ 19-21).



In β-dicarbonyls the carbon may become much more acidic
(*active methylene: pKa = 11-13 in β-diesters, 10-11 in β-ketoesters, down to 4-5 in β-diketones*).
This is due to the RAHB-driven enolization, where the enol form is strongly stabilized by an intramolecular O–H···O RAHB of high energy (12-20 kcal/mol).
The H-bond, moreover, contribute to stabilize the enolate intermediate by delocalizing the negative charge all over the H-bonded ring.



1. RAHB-driven prototropic tautomerism

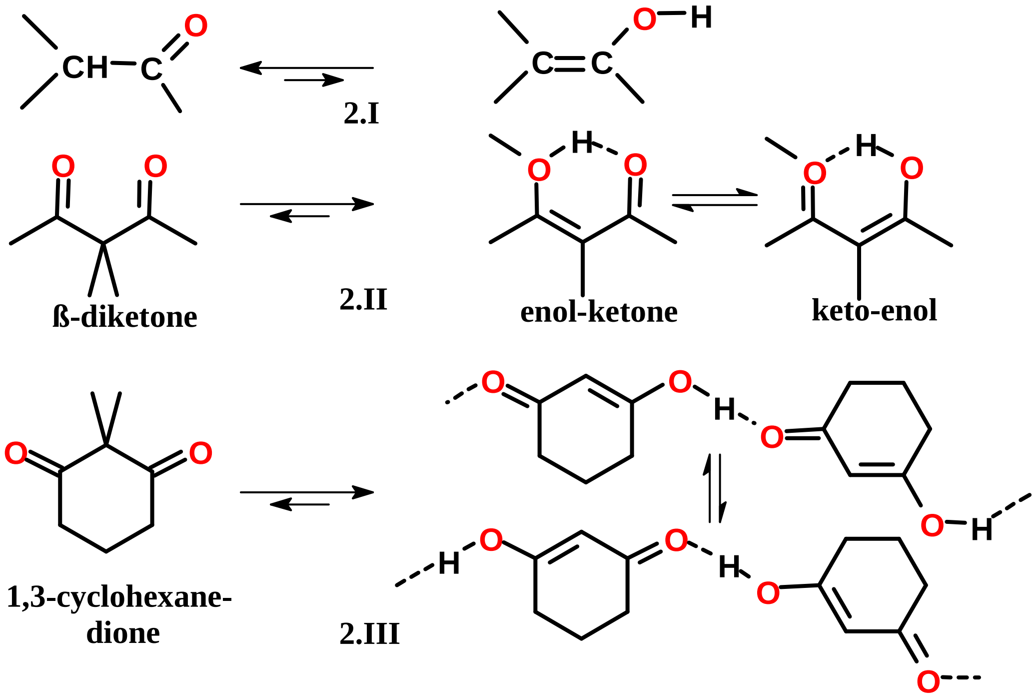
1.2 Keto-Enol and Keto-Enol↔Enol-Keto tautomerism

The keto-enol tautomerism is always strongly shifted to the left. However, the enolization is highly enhanced when the enol form can be supported by an *intramolecular* or *intermolecular* RAHB.

The enol produced undergoes further tautomerization through the Keto-Enol ↔ Enol-Keto equilibrium.

RAHB–DRIVEN KETO–ENOL TAUTOMERISM

KETO – ENOL TAUTOMERISM KETO/ENOL – ENOL/KETO TAUTOMERISM



1. RAHB-driven prototropic tautomerism

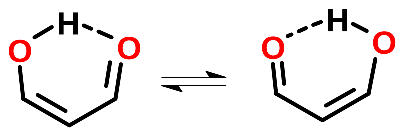
1.3 Prototropic tautomerism in hetero-conjugated systems

The great part of <u>prototropic tautomeric equilibria</u> in organic chemistry are Keto-Enol ↔ Enol-Keto equilibria.

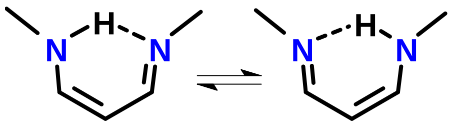
As a rule, they involve an

Heteroconjugated System

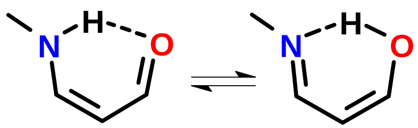
linked by a strong intramolecular RAHB.



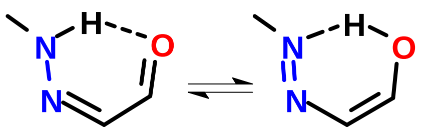
enol-ketone **keto-enol**



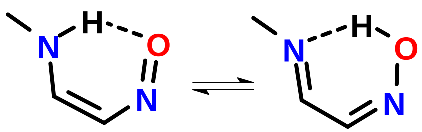
enamino-imine **imino-enamine**



enaminone **imino-enol**



keto-hydrazone **azoenol**



nitroso-enamine **imino-oxime**

Provisional Conclusion

Most probably, RAHB is <u>the first cause</u> of prototropic tautomerism in organic chemistry

1. RAHB-driven prototropic tautomerism

1.4 More complex examples of RAHB-driven tautomerism

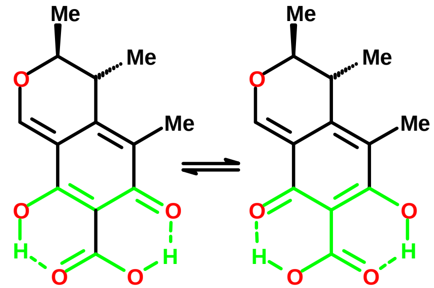
The list of RAHB-induced tautomeric systems is practically endless.

They can form, besides normal rings and chains,

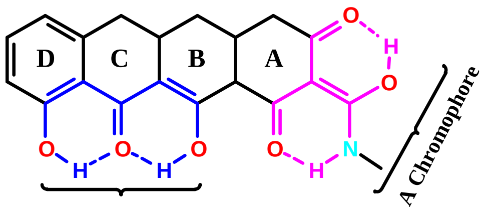
ring associations

which combine with mutual

π-Bond-Cooperativity or π-Bond-Anticooperativity Rules

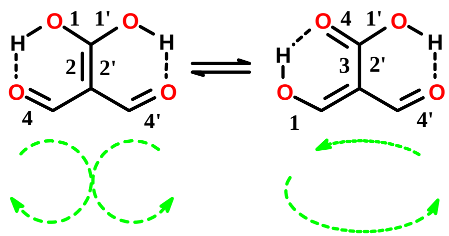


citrinin, a fungal metabolite



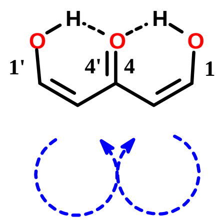
tetracyclines, antibiotics from *Streptomyces*

[2,2']OOOO **[3,2']OOOO**



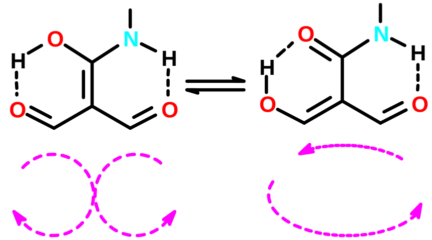
R_3/R_3 ANTI-COOP. **R_1+R_3 COOP.**

[4,4']OOO



R_3/R_3 ANTI-COOP.

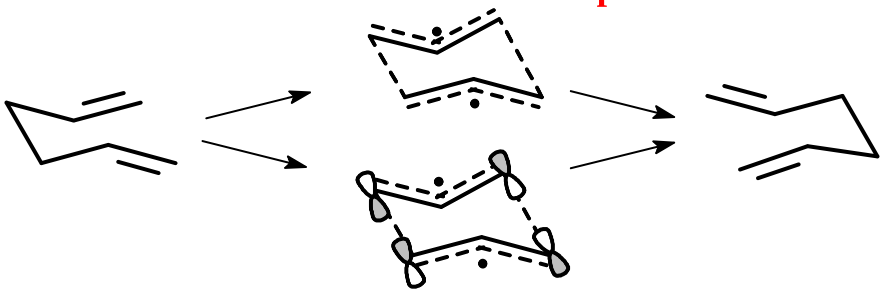
[2,2']OONO **[3,2']OONO**



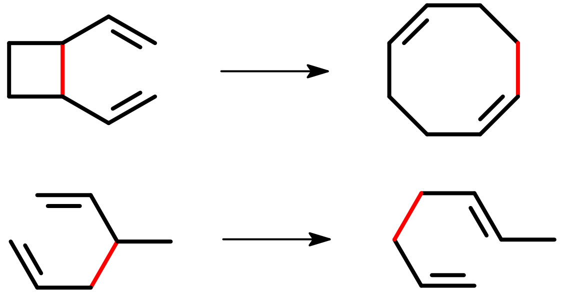
R_3/R_3 ANTI-COOP. **R_1+R_3 COOP.**

2. Some chemical and biochemical RAHB applications
2.1 RAHB-driven aza-Cope rearrangement

The **Cope Rearrangement** is an important organic reaction involving the **[3,3] sigmatropic rearrangement of 1,5-dienes**. Variations of the Cope rearrangement including heteroatoms a the **Oxo–Cope** and tha **Aza–Cope** rearrangements.



diradical transition state

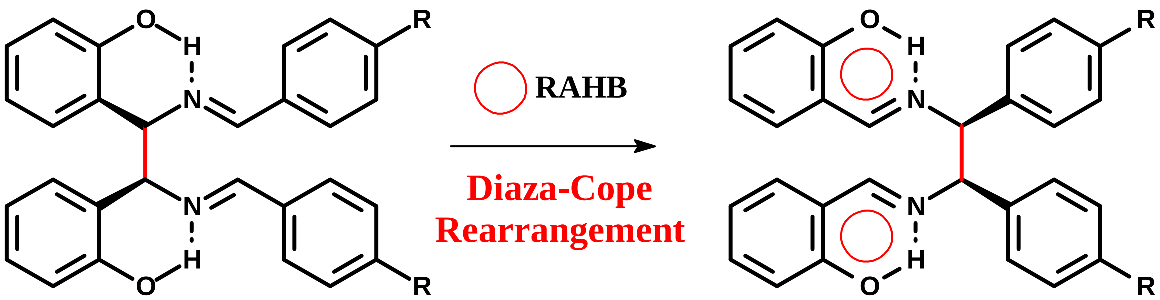


Controlling Diaza-Cope Rearrangement Reactions with Resonance-Assisted Hydrogen Bonds

J Chin, F Mancin, N Thavarajah, D Lee, A Lough, DS Chung, JACS 2003, 125:15276.

Following papers:

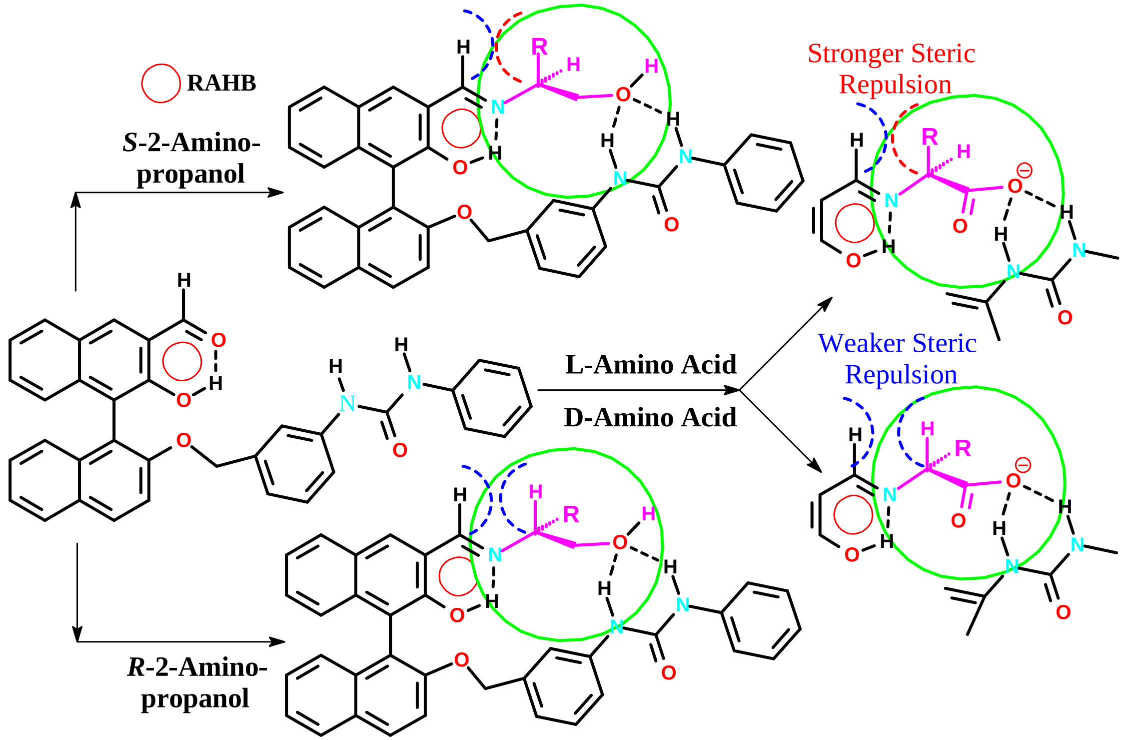
JACS 2005, 130:12184; AngewChem 2008, 47:8678; JOC 2009, 74:3330



2. Some chemical and biochemical RAHB applications
2.2 RAHB-driven enantiomeric recognition

Enantioselective Recognition of <u>1,2-Amino Alcohols</u> by Reversible Formation of Imines with <u>Resonance-Assisted Hydrogen Bonds</u>.

KM Kim, H. Park, H-J Kim, J Chin, W Nam, OrgLett 2005, 7: 3525. **Following papers: JACS 2007, 129: 1518; ChemEurJ 2008, 14: 9935; AngewChem 2008, 47:8678.**



Roles played by RAHB in the sensor–ligand complex

a) Thermodynamic stabilization of the imino complex;

b) Planarization of the complex by the intramolecular RAHB ring

c) Consequent discrimination of the <u>^{1}H NMR signals</u> because of the different steric strain induced by the two enantiomers

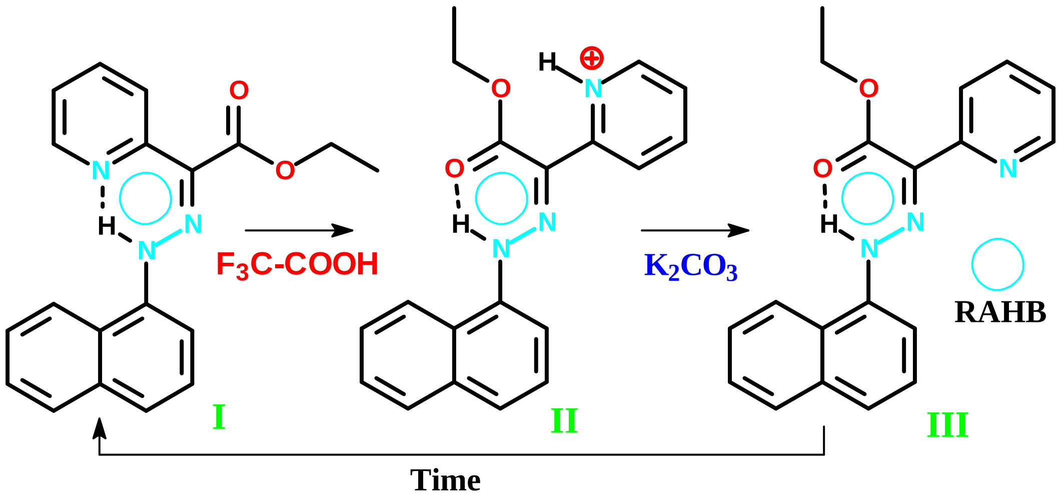
2. Some chemical and biochemical RAHB applications
2.3 A RAHB-driven configurational rotary switch

Working mechanism of the rotary switch

1. **Protonation of I at the pyridine N atom causes the breaking of the N–H···N RAHB, rotation of the group, and the formation of the new N–H···O RAHB in II;**
2. **Deprotonation of I to II in alkali leaves the N-H…O RAHB unaltered. Since, however, heteronuclear RAHBs are weaker than homonuclear ones, with the time III slowly returns to the thermodynamically more stable form I.**

A pH Activated Configurational Rotary Switch. Controlling the *E/Z* Isomerization in Hydrazones

SM Landge, I Aprahamian, JACS 2009, 131:18269.

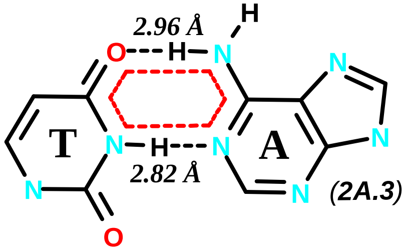


2. Some chemical and biochemical RAHB applications
2.4 RAHB in DNA base pairing and secondary protein structure

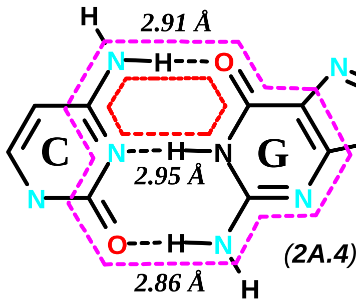
In our first paper on the intermolecular RAHB [G Gilli, V Bertolasi, V Ferretti, P Gilli, ActaCryst 1993, B49:564] we wrote: *"nature itself may have taken advantage of the greater energy of RAHB to keep control of molecular associations whose stability is essential to life"*.

This statement was grounded of two classes of observations:

1. In DNA coupling, both Thymine-Adenine and Cytosine-Guanine couples are linked as RAHB dimers and the C-G coupling includes a further much wider cycle of resonant HB.

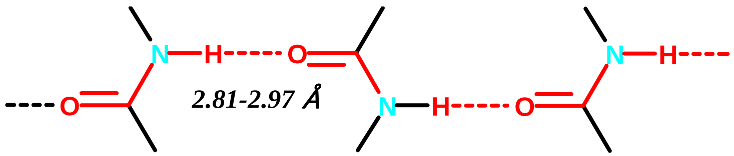


(2A.3)



(2A.4)

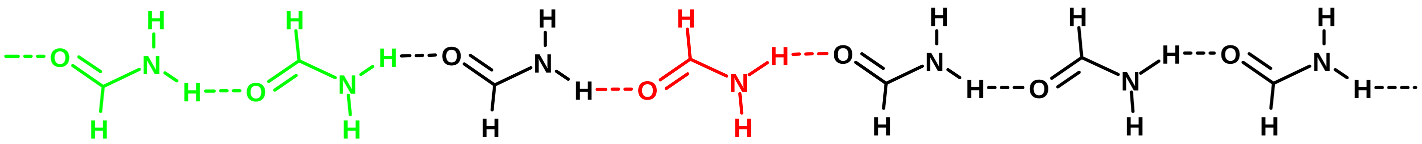
2. In the secondary structure of proteins, residues are linked in form of R_1-RAHB chains:
α-helices contain three nearly parallel and isooriented RAHB chains and
β-pleated sheets infinite parallel or antiparallel arrays of such chains



2. Some chemical and biochemical RAHB applications
2.4 (cont) RAHB in DNA base pairing and secondary protein structure

1. **RAHB in DNA Base-Pairing has been object of many studies without coming, so far, to any convincing conclusion, probably because the H-bonds formed are <u>too weak</u> and then too <u>easily perturbable</u> by the solvent and by the environment.**

2. **Also RAHB in amide-chains is <u>too weak</u> to be definitely assessed by crystal methods. It has been, however, convincingly confirmed by the QM studies carried out by *Kobko & Dannenberg (JPhChem, 2003)* at the DFT-B3LYP/D95(d,p) level:**



	E(HB) kcal/mol	*d*(NH...O) Å	*d*(C=O) Å	*d*(C−N) Å	μ Debye
Dimer	**4.50**	**1.92**	**1.225**	**1.360**	**<4.6>**
Center of 15	**13.00**	**1.78**	**1.293**	**1.344**	**<5.9>**
Δ%	**+189%**	**–7 %**	**+6**	**–1.2**	**+28%**

According to RAHB predictions, the increasing number of residues in the chain induces:

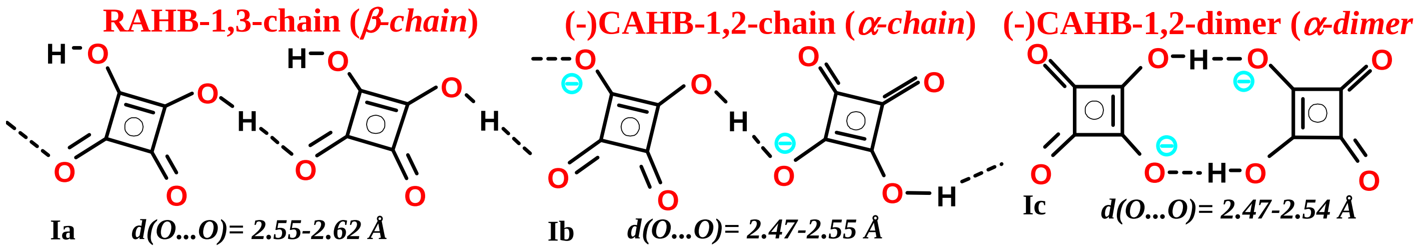
1. A moderate NH...O shortening and a small increase of amide π-delocalization (i.e., C–N shortening and C=O lengthening);

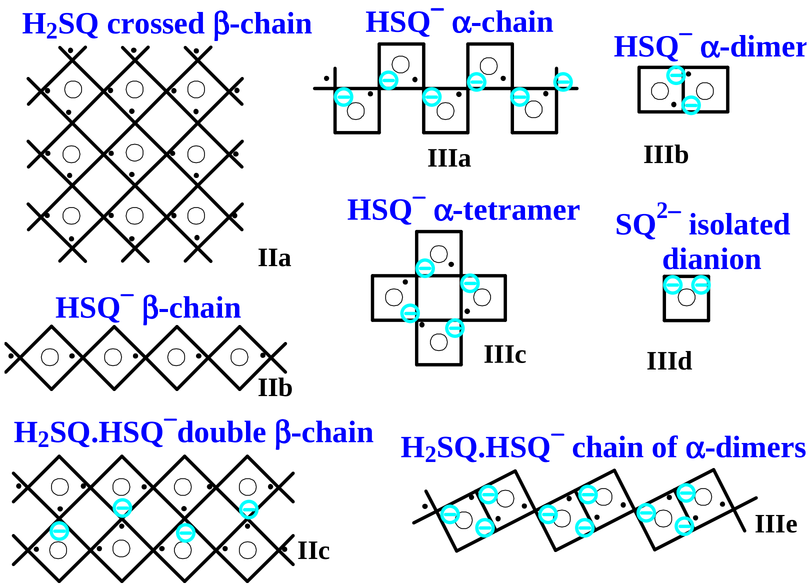
2. A very large increase (nearly three-times) of the H-bond energy ;

3. A large increase of the dipole moment/residue (for which an α-helix of ten residues would have a dipole moment as high as 59 Debye)

3. Strong RAHB- and CAHB-controlled crystal packing
3.1 The crystal packing of squaric acid and its anions

Squaric acid and its anions are characteristic for forming *three different patterns of strong HBs*, one controlled by *RAHB* and two by *(–)CAHB*.





All these patterns are found in molecular crystals, both alone and in combination, giving rise to a variety of *2-D combination patterns* which are the *topological equivalent to the 3-D patterns typical of the orthosilicic acid in silicate minerals*.

In a sense, squaric acids could be considered

The Silicates of Flatland

4. Bistable H-bonds in functional molecular materials

4.1 The nanoscale mechanism of functional materials

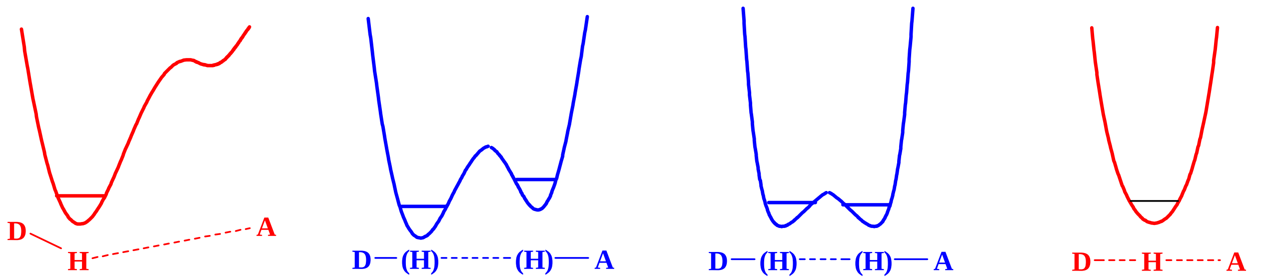
Functional materials are systems performing specific functions at atomic (or nanoscale) level. They have important technological applications in electronics, optoelectronics, photonics, memory storage, signal transmission and amplification, sensors, and so on.

They can be thought of as assemblies of nanoscale molecular machines (molecular devices), each *based on a* two-state (bistable) molecular system operating by *cyclically switching between the two different stability points*. The switching force can arise from:

energy transfer, charge (or electron) transfer, and proton transfer (PT).

In particular, H-bond-driven functional materials can be defined as assemblies of bistable molecular systems based on tautomeric H-bonds.

This immediately points to strong H-bonds with a double-well (DW) PT profile and small PT barrier (LBHBs). W*eak* (OHBs) or *very strong* (single-well, SW) bonds are of no use because their having too high or too low PT barrier.



aSW-HB — Asymmetric Single-Well High-Barrier

aDW-MB — Asymmetric Double-Well Medium-Barrier

sDW-LB — Symmetric Double-Well Low-Barrier

sSW-NB — Symmetric Single-Wel No-Barrier

4. Bistable H-bonds in functional molecular materials
4.1(cont) The nanoscale mechanism of functional materials

Of course, <u>not all</u> tautomeric H-bonds <u>are suited</u> to build a nanoscale molecular machine.

To this aim, they must possess another and much less common property: the proton shift must be able to reverse the value of some physical quantity of the system.

This quantity will then become the <u>steering variable</u> for switching the system itself, from the outside, from one state to the other.

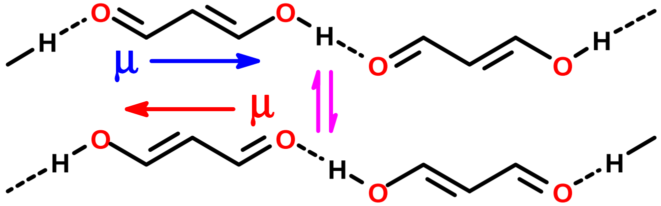
Such a <u>steering variable</u> can be of quite a different nature, such as electric or magnetic fields, pressure, temperature, or irradiation in a proper frequency range.

Two basic examples are illustrated below:

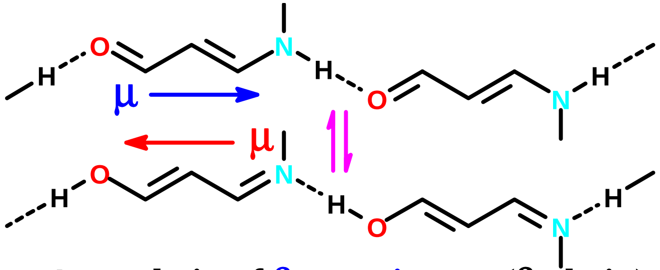
In ferroelectric crystals the <u>property of interest</u> is the electric dipole moment and the <u>steering variable</u> is an external electric field.

In molecules undergoing excited-state proton transfer (ESPT) the H-bond assumes tautomeric configurations (X−H···Y or X···H−Y) according to whether the molecule is in its ground or excited state.
Accordingly, the <u>steering variable</u> comes from irradiation by a suitable wavelength.

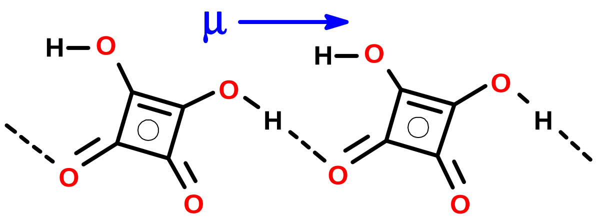
4. Bistable H-bonds in functional molecular materials
4.2 RAHB and ferro/antiferroelectric behavior



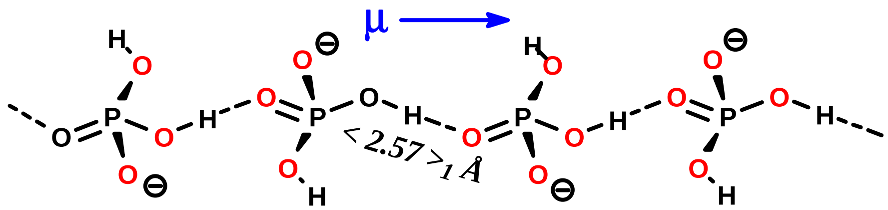
RAHB chain of β-enolones (β-chain)



RAHB chain of β-enaminones (β-chain)



RAHB chain of squaric acids *(*β-chain*)*



RAHB Chain of Dihydrogen Phosphates (β-chain)

β-chains are tautomeric RAHB-bonded chains of β-enolones giving rise to a bistable system having inverted dipole moment of the chain.

They are prototypic constituents of ferroelectric crystals, molecular crystals able to display ferroelectic/antiferroelectric ordering below their critical or Curie temperature and order-disorder transition to paraelectric beyond it.

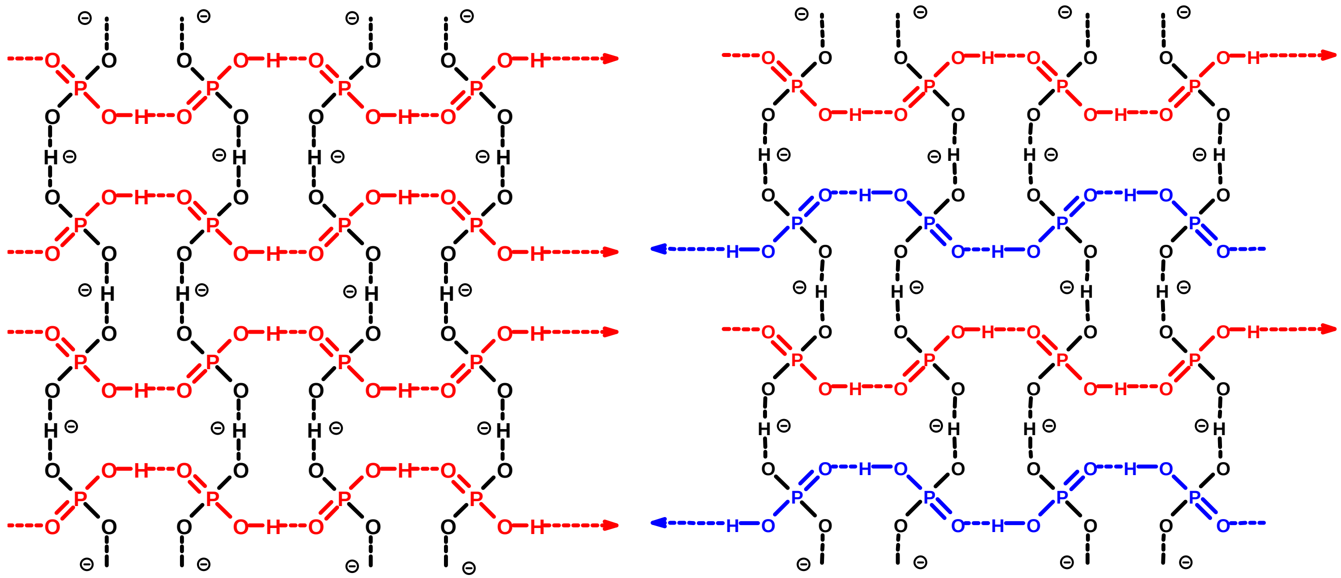
4. Bistable H-bonds in functional molecular materials
4.2(cont) RAHB and ferro/antiferroelectric behavior

Order-disorder and displacive ferroelectrics are important functional materials without which medical ultrasounds, sensors, sonars, compact laptops, ferroelectric RAM, cell phones, and electro-optic modulators at the base of internet would not exist.

RAHB plays a basic role in *order–disorder ferroelectrics*, such as ferroelectric KDP (K dihydrogen phosphate): T_C = 213 K; and antiferroelectric ADP (ammonium dihydrogen phosphate): T_C = 230 K.

Being disordered above the critical temperature (Tc), they become ordered below Tc and, at lower temperatures, the ordering becomes complete with perfect orientation of all dipoles in the same direction (*ferroelectric crystals*) or in alternating directions (*antiferroelectric crystals*).

In ferroelectic KDP, in particular, application of a suitable electric potential will be able to reverse the direction of all dipoles, at a time. The mechanism of non-volatile FeRAM Memories.



KH_2PO_4 (KDP)
Ferroelectric Phase

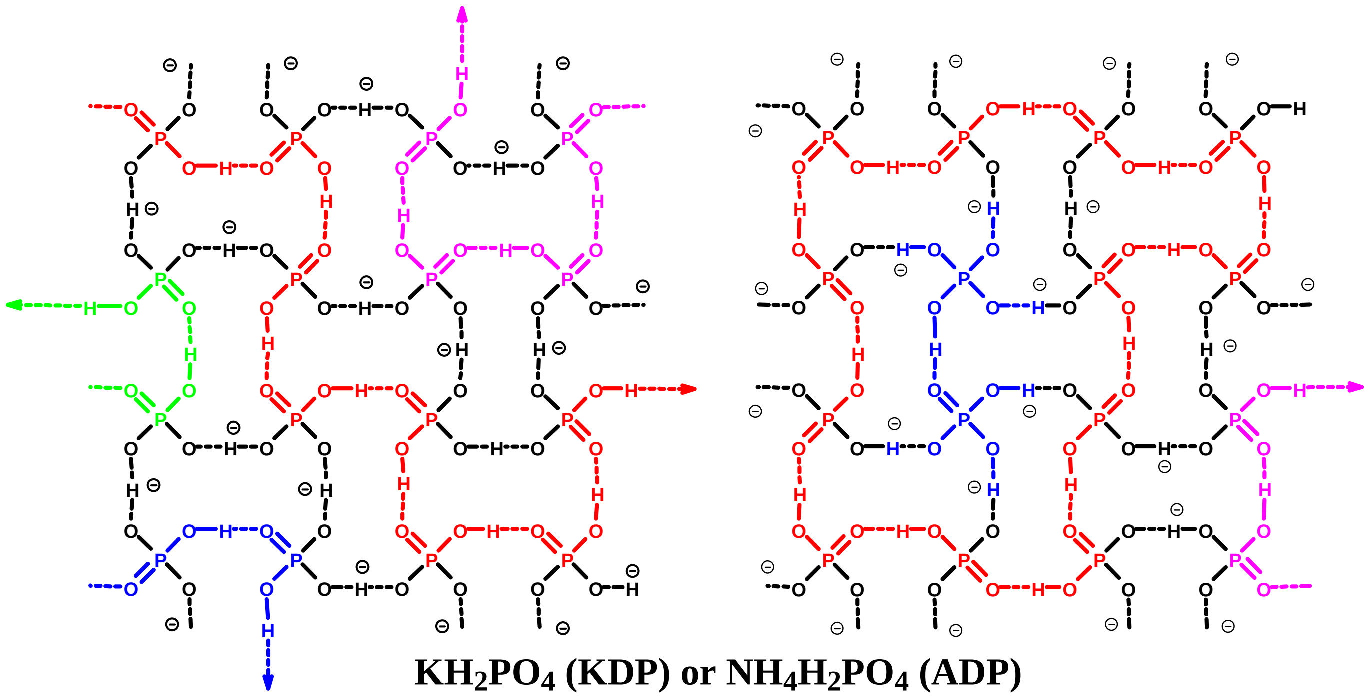
$NH_4H_2PO_4$ (ADP)
Antiferroelectric Phase

4. Bistable H-bonds in functional molecular materials
4.2(cont) RAHB and ferro/antiferroelectric behavior

A. ferroelectric KDP (potassium dihydrogen phosphate): T_C = 213 K;
B. antiferroelectric ADP (ammonium dihydrogen phosphate): T_C = 230 K).

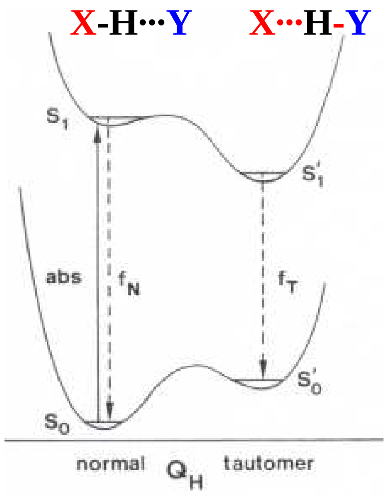
When KDP and ADP reach their <u>critical temperature (*Tc*)</u> an order-disorder transition occurs. The ferroelectric or antiferroelectric order disappears, substituted by a critical state where the electric dipoles may assume all possible orientations.

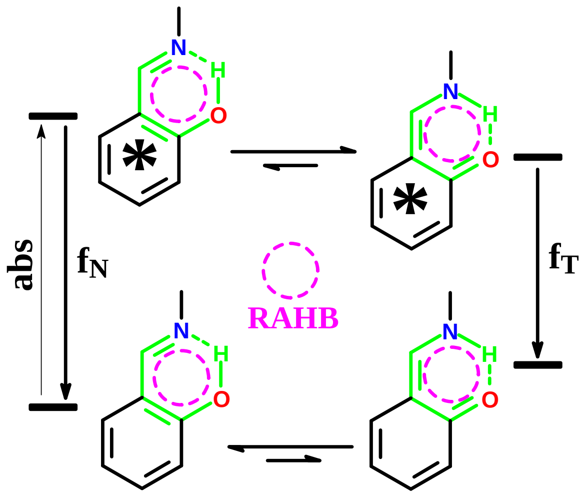
But: *How can be the critical states of KDP and ADP, whose structure consist of infinite chains interlinked in a 2-D lattice?* The schemes below try to represents two of the <u>infinite disordered arrangements</u> associated with this critical state.



KH_2PO_4 (KDP) or $NH_4H_2PO_4$ (ADP)
Two Critical-State Configurations

4. Bistable H-bonds in functional molecular materials
4.3 RAHB and photo-induced excited-state proton transfer (ESPT)





Excited-state proton transfer (ESPT) **occurs when** **the two minima of the PT-pathway have reversed stabilities in the *ground* (lower curve) and *excited state* (upper curve).**

Excitation of the X−H···Y ground-state configuration S_0 to its excited state S_1 may be followed by adiabatic PT with formation of the excited-state X···H−Y tautomer S'_1.

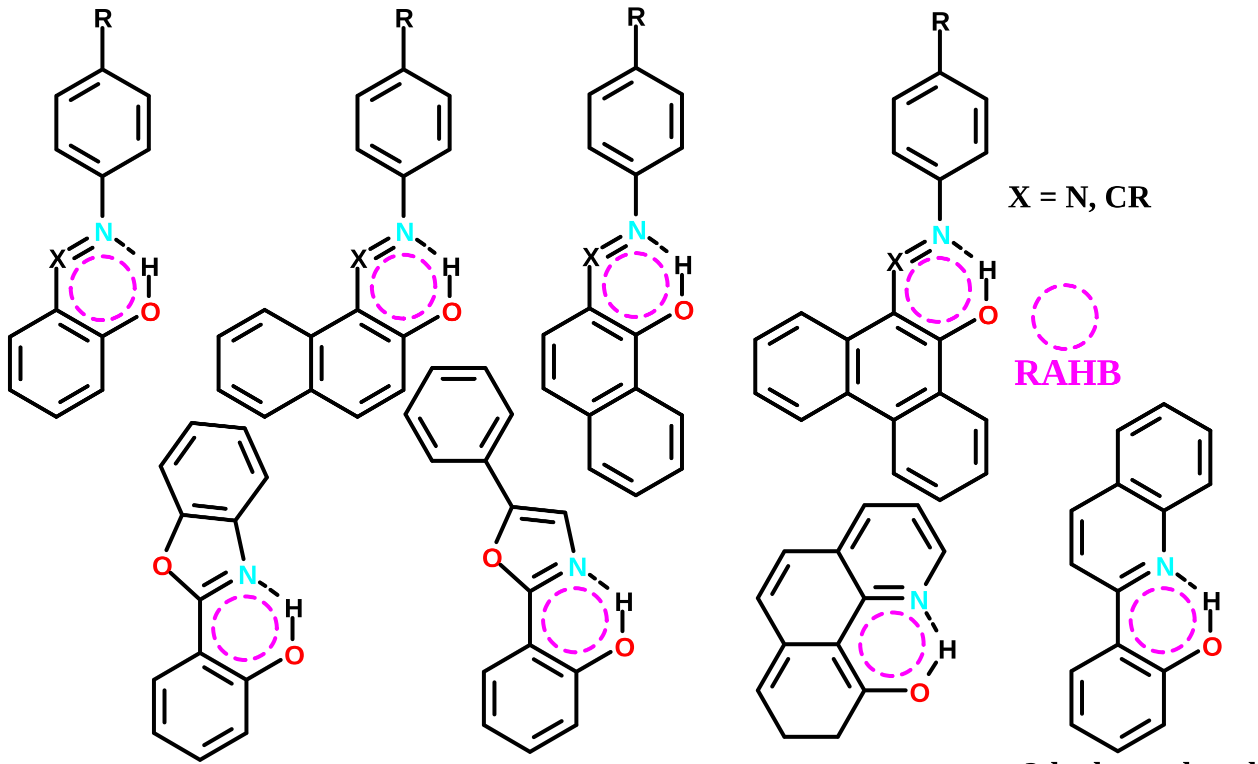
An interesting case of ESPT is the RAHB-controlled ESPT (see figure). In the ground state, the tautomeric O–H···N ↔ N–H···O equilibrium is shifted to the left by the phenyl resonance energy. The π* ← π excitation, spoiling such resonance energy, inverts the stability order of the two H-bonded forms.

4. Bistable H-bonds in functional molecular materials

4.3(cont) RAHB and photo-induced excited-state proton transfer (ESPT)

Not surprisingly, these molecules may be thermochromic, photochromic and photoluminescent and represent a good starting point for the design of a variety of molecular devices, such as dye-lasers, energy storage, optical-data storage, solar collectors, optical switches, luminescent probes, scintillators, and proton-transfer materials.

RAHB-controlled ESPT. A small number of possible examples is shown below.



2-hydroxyphenyl-benzoxazole

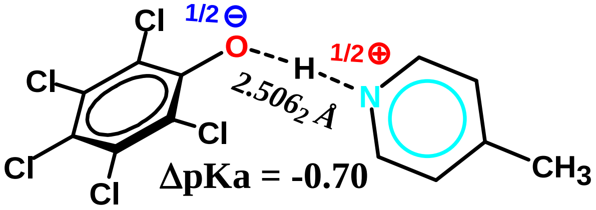
2-hydroxyphenyl-5-phenyloxazole

10-hydroxybenzo[*h*]quinoline

2-hydroxyphenyl quinoline

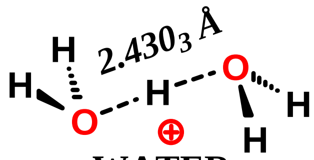
5. Charge-assisted H-bonds (CAHBs)

CL # 1: (±)CAHB ⇒ S, VS
Double Charge-Assisted HB
Acid-Base



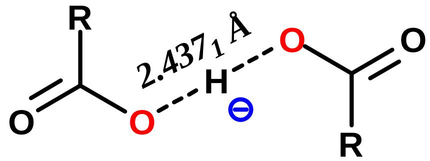
PENTACHLOROPHENOL - *p*-TOLUIDINE

CL # 2: (–)CAHB ⇒ S, VS
Negative Charge-Assisted HB
Acid-Acid



WATER - HYDRONIUM

CL # 3: (+)CAHB ⇒ S, VS
Positive Charge-Assisted HB
Base-Base



CARBOXYLIC ACID - CARBOXYLATE

D–H···A		E_{HB}	E_{HB}
[F···H···F]⁻	–	≈ 6	42(3)
[O···H···O]⁻	–	≈ 5	27(1)
[Cl···H···Cl]⁻	–	≈ 2	24(3)
[N···H···N]⁻	–	≈ 3	12(-)
[Br···H···Br]⁻	–	≈ 2	19(3)
[S···H···S]⁻	–	≈ 1	13(1)
[F···H···F]⁺	+	≈ 6	25(2)
[O···H···O]⁺	+	≈ 5	32(2)
[N···H···N]⁺	+	≈ 3	26(2)
[O···H···O]±	±	≈ 6	*28.7*
[N···H···O]±	±	≈ 4	15.2
[N···H···N]±	±	≈ 3	*16.4*

CAHBs are among the most important types of strong H-bonds.

CAHBs play a primary role in many Functional H-Bonds through a mechanism of H-bond-strength modulation induced by changes of the acid/base properties of the environment.

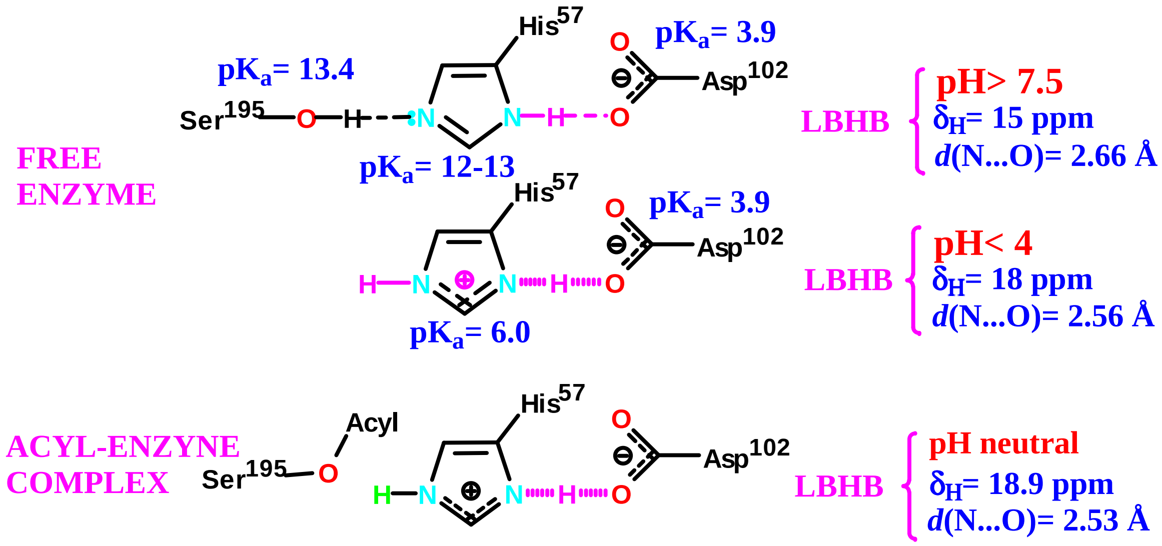
CAHB most known applications concern a number of mechanisms of enzymatic catalysis, where they are known to cause remarkable stabilization of the transition state by forming a very strong type of H-bond, usually called LBHBs (Low-Barrier Hydrogen Bonds).

5. Charge-assisted H-bonds (CAHBs) in enzymatic catalysis
5.1 (±)CAHB in the catalytic mechanism of serine proteases

Generalities. **The hypothesis that short, strong, low-barrier H-bond (LBHB) may play an important role in enzymatic catalysis was proposed in the early '90s (Cleland, 1992; Gerlt and Gassman, 1993; Cleland and Kreevoy, 1994: Frey *et al*., 1994) based on new high-resolution NMR techniques able to detect strong H-bonds inside the enzymatic binding site.**

The first case studied was the reaction mechanism of serine proteases, an important class of proteolitic enzymes such as trypsin, chymotrypsin or subtilisin.

The Catalytic Triad. **The active site includes three residues: *serine*, *histidine*, *and aspartate*. The clue of the mechanism is hystidine that displays two different pK_a values (12-13 or 6.0) in neutral or acidic environment (pH>7.5 or pH<4) and then can H-bind serine or aspartate according to the situation.**

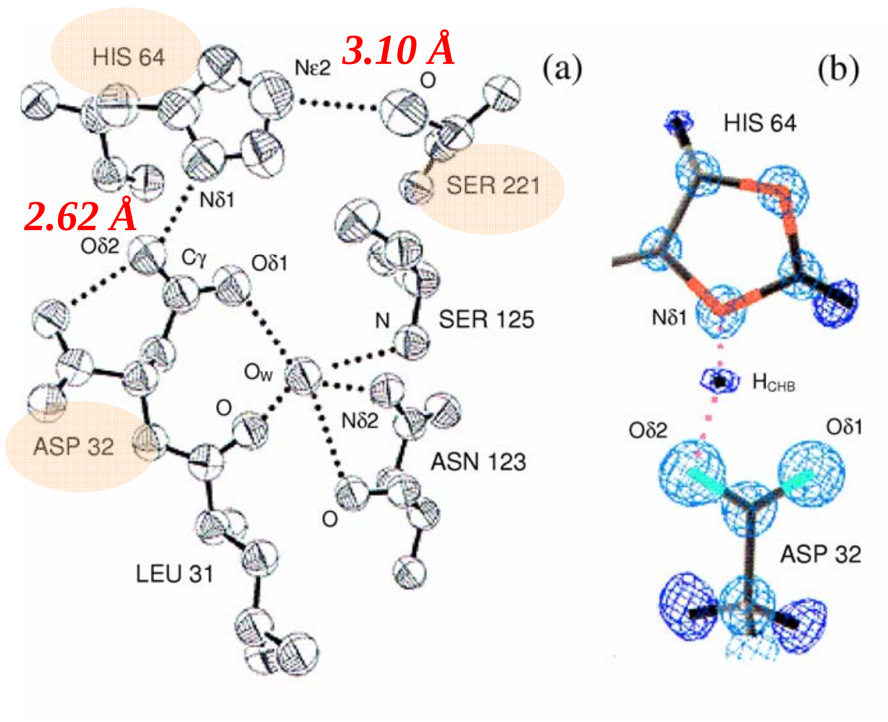


5. Charge-assisted H-bonds (CAHBs) in enzymatic catalysis
5.1 (cont) (±)CAHB in the catalytic mechanism of serine proteases

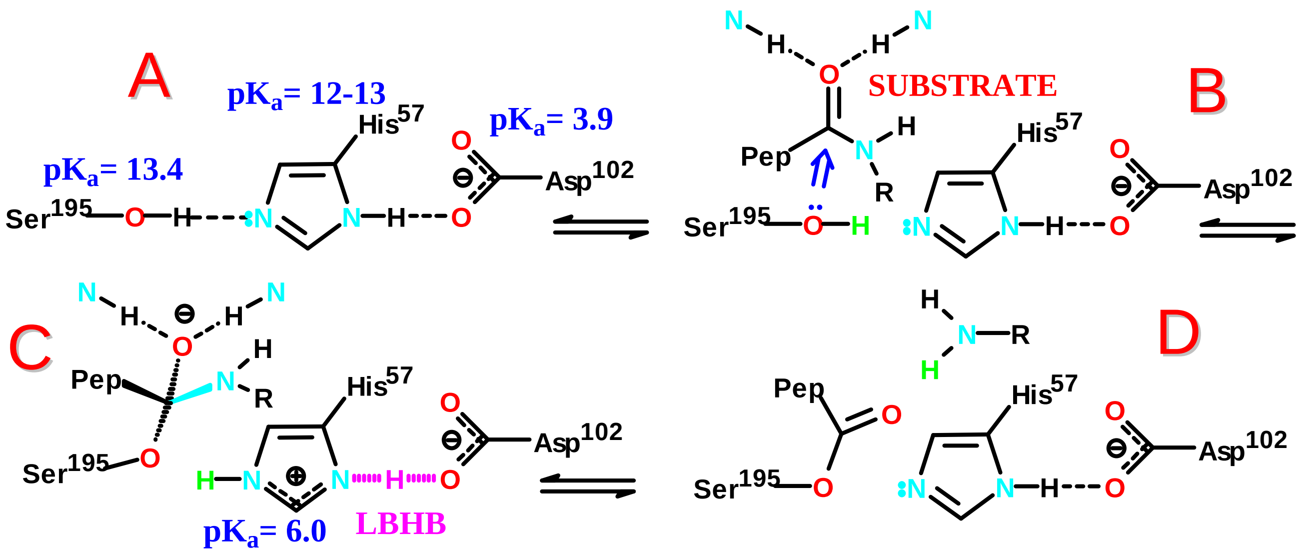
The crystal structure of serine protease *Bacillus lentus* subtilisin as determined by X-ray diffraction at 100 K and 0.78 Å resolution (Reproduced by permission from Kuhn *et al*., Biochemistry 1998, 37: 13446).

(a) The three Ser221, His64, and Asp32 residues building up the catalytic triad are interlinked by two H-bonds having quite different N···O distances, 2.62 Å for His-Asp and 3.10 Å for Ser-His;

(b) the difference-Fourier electron density map has identified a small peak at 1.2 Å from N*δ*1 and 1.5 Å from O*δ*2 to be associated with the proton of the N−H···O bond.



5. Charge-assisted H-bonds (CAHBs) in enzymatic catalysis
5.1 (cont) (±)CAHB in the catalytic mechanism of serine proteases



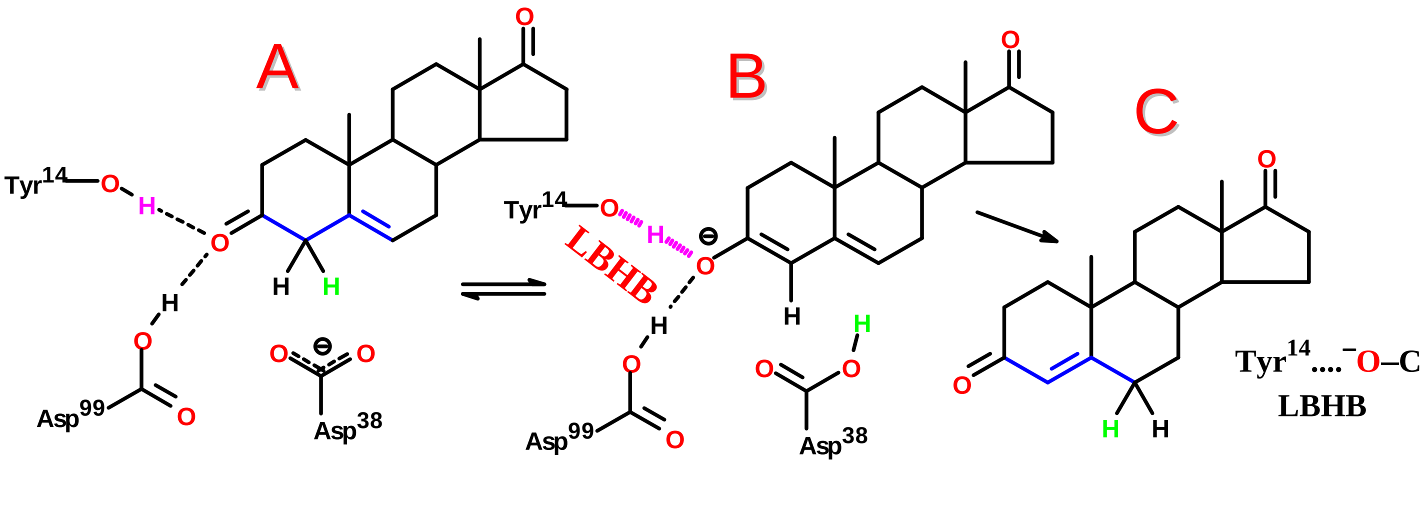
The mechanism by which serine proteases cut the protein chains is a two-step process, out of which only the first (A-D) is of interest here.

The peptide bond is cleaved by nucleophilic addition of serine to the substrate (B) to form a tetrahedral acyl-enzyme intermediate (C) which will then release the amine product (D).

In its mechanism of action the very strong LBHB [or (±)CAHB] is supposed to play the double function of catalysing serine deprotonation (B) and of stabilizing the transition state (C) by lowering its energy (Frey, 2001, 2002).

5. Charge-assisted H-bonds (CAHBs) in enzymatic catalysis

5.2 (─)CAHB in the catalytic mechanism of Δ^5-3-ketosteroid isomerase (KSI)

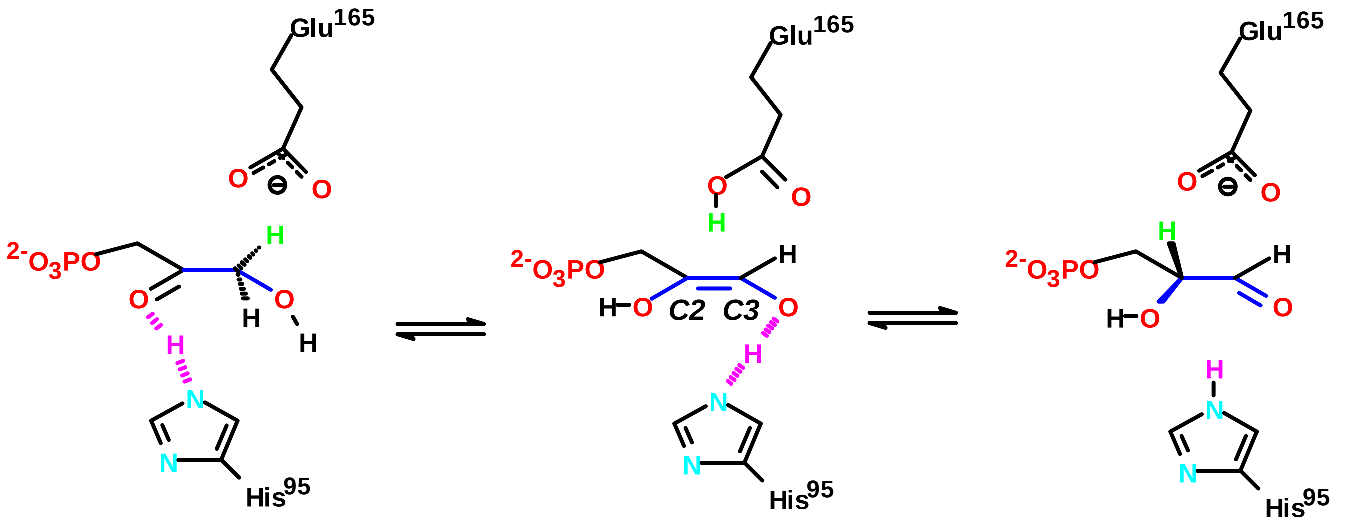


δ_H = 18.2 ppm
ϕ = 0.34
$d(\text{O...O})_{CALC,\delta} = 2.49_2$ Å
$d(\text{O...O})_{CALC,\phi} = 2.50_1$ Å
$d(\text{O...O})_{EXP,X} = 2.58_{23}$ Å

Ketosteroid isomerase (KSI) catalyses the conversion of Δ^5- or Δ^4–3-ketosteroids using <u>Asp38</u> as a general base and both <u>Tyr14</u> and <u>Asp99</u> as general acids.

The transition state (B) is stabilized by a strong [O···H···O]⁻ bond to be classified as a Low-Barrier H-Bond (LBHB) of Negative Charge-Assisted H-Bond [(─)CAHB].

5. Charge-assisted H-bonds (CAHBs) in enzymatic catalysis
5.3 CAHBs in the catalytic mechanism of triosophosphatoisomerase (TIM)



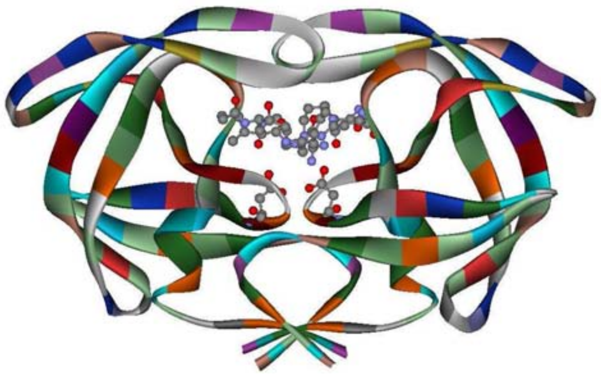
Triosophosphato-isomerase (TIM)
catalyzes the conversion of di-hydroxy-acetone phosphate (DHAP) to a *cis*-enediolic intermediate.

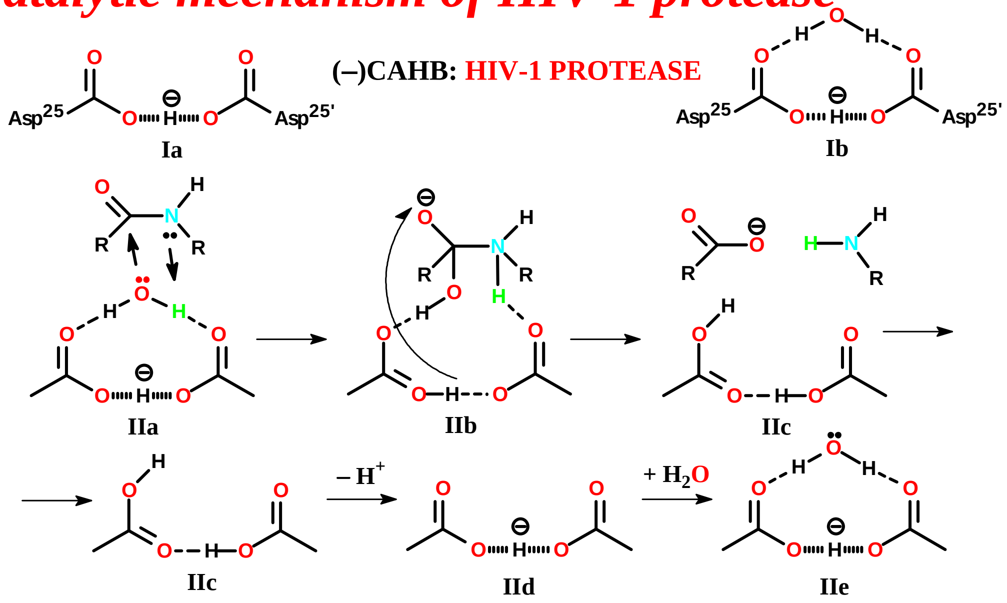
The enediolic intermediate results from the abstraction of the C_3 proton by Glu165, facilitated by polarization of the C_2=O carbonyl by the LBHB it forms with His95.

QM/MM calculations by: **PA Bash, MJ Field, RC Davenport, GA Petsko, D Ringe, M Karplus.** ***Computer simulation and analysis of the reaction pathway of triosephosphate isomerase.*** **Biochemistry 1991, 30: 5826.**

5. Charge-assisted H-bonds (CAHBs) in enzymatic catalysis

5.4 (–)CAHB in the catalytic mechanism of HIV-1 protease





HIV-1 protease (human immunodeficiency virus protease) is an aspartic protease that performs an essential step in the life cycle of HIV virus, the etiologic agent of AIDS. Its inhibitors are important drugs in AIDS treatment because they can block virus maturation.

HIV-1 consists of two identical domains of 99 residues, assembled to form a tunnel which accommodates the protein to be cleaved (or the inhibitor) and linked by a very strong aspartate-aspartic acid [O···H···O]$^-$ (–)CAHB.

The reaction centre is the water-aspartate-aspartic acid complex (IIa): during the reaction water adds to the substrate (IIb, IIc) causing the hydrolysis of the peptide bond. After dissociation of the products (IIc), the original reaction centre is restored (IIe).

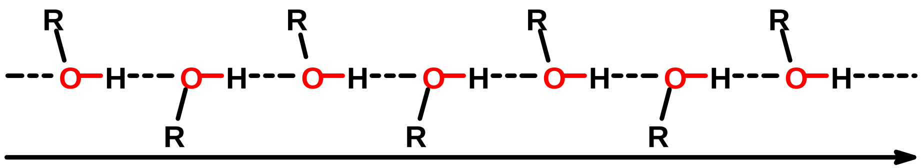
The reaction is a case of general basic catalysis where the negatively charged <u>aspartate enhances the nucleophilic character of water through H-bond formation</u>.

6. Σ-bond cooperativity and anticooperativity in PAHBs
6.1 Cooperative and anticooperative water chains

The phenomenon by which a combination of HBs displays larger binding energy than the sum of the constituting H-bonds is called *H-Bond-Cooperativity*. Only two chemical leitmotifs can produce H-bond-cooperativity:

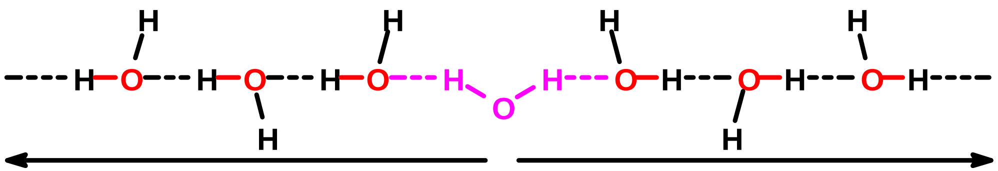
RAHB (Resonance-Assisted HB) → π-Bond-Cooperativity → large π-bond polarizability
PAHB (Polarization-Assisted HB) → σ-Bond-Cooperativity → small σ-bond polarizability

σ-Bond-Cooperativity is normally associated with *the* formation of *homodromic chains (or cycles)* of O–H···O bonds (R= alkyl, aryl or H in alcohols, phenols or water, respectively).



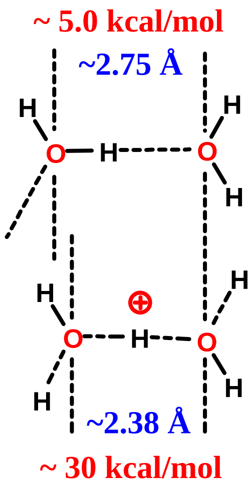
Though σ-bond polarizability is rather smal, O–H···O PAHBs can still provide H-bond-energy enhancements of 20-45% with a O···O shortenings from 2.75 to 2.62 Å, which are sufficient to produce a large number of interesting physical effects.

Homodromic σ-bond-cooperative chains of waters can be interrupted by a local defect which reverses the chain polarity through a single point of σ-bond-anticooperativity.



6. Σ-bond cooperativity and anticooperativity in PAHBs

6.2 Fast proton transmission in water



The **mechanism of proton transmission in water** is based on the combination of two different Chemical Leitmotifs, ***PAHB** and **(+)CAHB***.

PAHB **provides the *proton wire* of *σ-bond-cooperative ...O-H...O-H...O-H... bonds* along which the proton travels.**

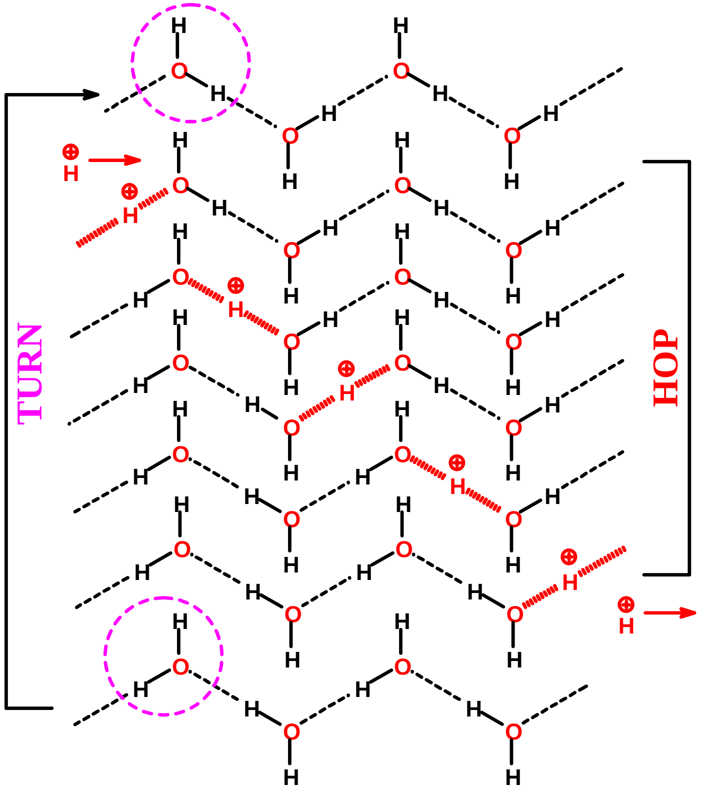
(+)CAHB **gives the *traveling soliton* consisting of a very short and energetic [O...H...O]$^+$ bond.**

The mechanism was firstly proposed by Grotthuss in 1806 and is known as the *“hop-and-turn”* or *“relais” Grotthuss mechanism*.

It consists in the V-driven transmission of the positive charge from left to right in the form of a *phonon vibration induced by the traveling soliton*.

Transmission is fast because <u>no proton moves</u> but the H-bond shift causes a rotation of all waters (the hop) . The rate-determining step is the final counter-rotation to restore waters in their initial positions again (the turn).

The mechanism assures a proton a mobility six times greater than that of any other ion in aqueous solutions.



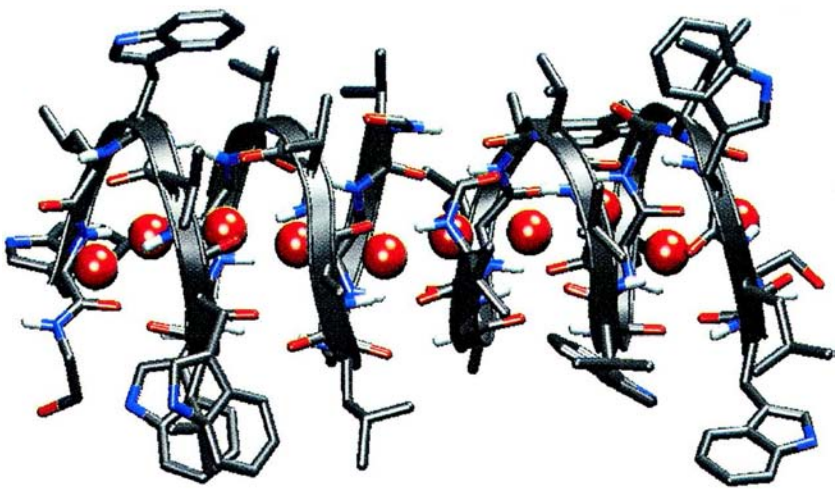
The *"Hop & Turn"* or *"Relais"* Grotthuss Mechanism
C.J.T. de Grotthuss, *Ann. Chim. LVII*, 54 (**1806**)

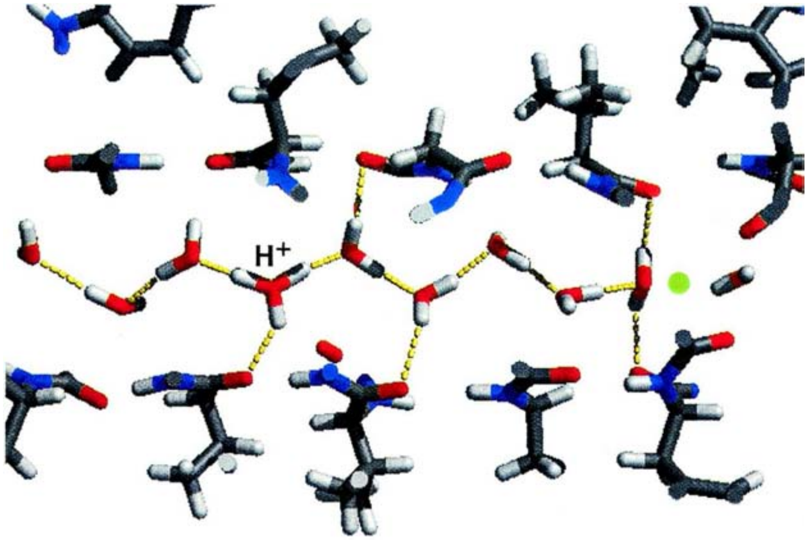
6. Σ-bond cooperativity and anticooperativity in PAHBs
6.3 Cooperative water transmission in gramicidin A channels

Proton conducting water chains are known as *Proton Wires* and have found important applications in explaining a number of molecular mechanisms, from conduction in carbon nanotubes to proton transmission in enzymatic mechanisms and in biological channels crossing the cell membrane.

A good example of transmembrane channel is: The Gramicidin A (gA) Channel.

Gramicidins are penta-deca-peptide antibiotics active against gram-positive bacteria by injecting protons (ions) through the conducting channels they are able to build up across the bacterial membrane. The channel consists of alternating L- and D-aminoacids arranged in β-helices making cylindrical pores that can accommodate a single chain of water molecules or of alternating waters and cations (figures from the molecular dynamic simulation carried out by Pomès & Roux, Biophys J 2002).





6. Σ-bond cooperativity and anticooperativity in PAHBs
6.4 Anticooperative water-without-proton transmission in aquaporin channels

To notice that, in the Gramicidin A example, the proton (ion) transmission across the bacterial membrane is a very efficient way to kill the cell itself (in this case the bacteria).

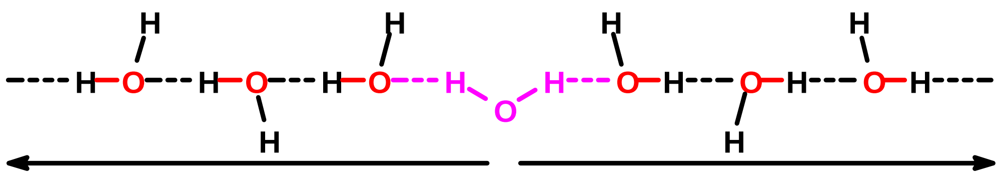
This suggests an intriguing problem:

Transmembrane Water Channels (Aquaporins) provide normal water flow through cell membranes and must therefore be able to assure water permeability without proton transmission because any even small change of the electrochemical potential across the cell membrane would kill the cells of any living organism (bacteria, plants, animals, and humans).

How to do that?

We will see that nature has solved the problem by using a trick we already know, that is:

two antidromic chains of waters inverted by a single anticooperative defect in the middle.



6.4 Anticooperative water-without-proton transmission in aquaporin channels (continued)

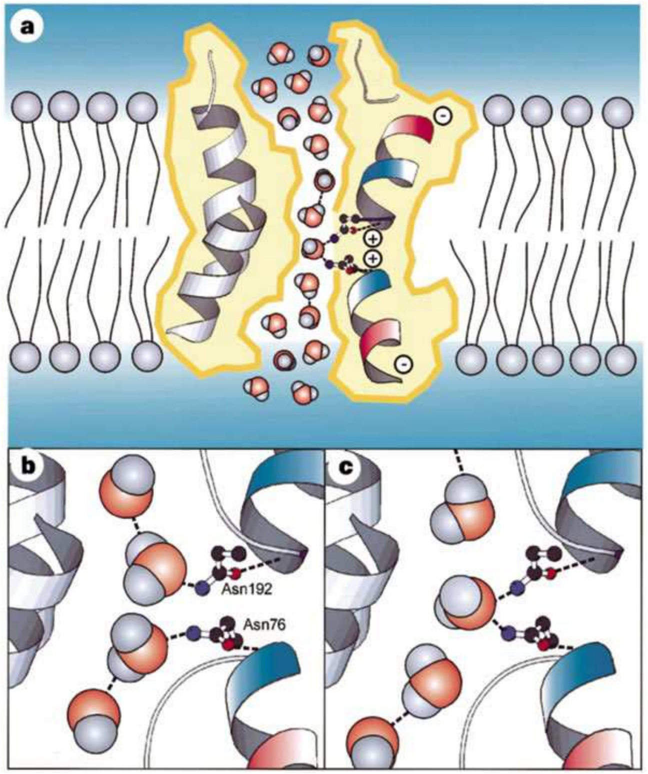
Membrane proteins deputed to form water-specific membrane-channels were firstly discovered in red blood cells and called *aquaporin-1* (*AQP*1: Preston, Carrol, Guggino, Agre, Science 1992, 256:385).

In the following years many other aquaporins were identified in animals and plants. Members of the aquaporin family have been found in archea, eubacteria and eucaryotes, where they serve an astonishing variety of physiological functions and are easily identified by their sequence similarities across all kingdoms of life (Tönroth-Horsefield *et al.*, Nature 2006, 439:688).

The drawing shows a scheme of the structure of aquaporin-1 embedded in the cell membrane (Murata et al., Nature 2000, 407:599), cut along the seven α-helices at the eight of the central water channel.

The partial charges from the helix dipoles restrict the orientation of the waters passing through the pore in opposite directions in the two halves of the chain.

The inversion of the water-chain direction is caused by the simultaneous H-binding of the central water to the two asparagine residues (Asn76 and Asn192), so introducing a single point of σ-bond anticooperativity in the chain itself.



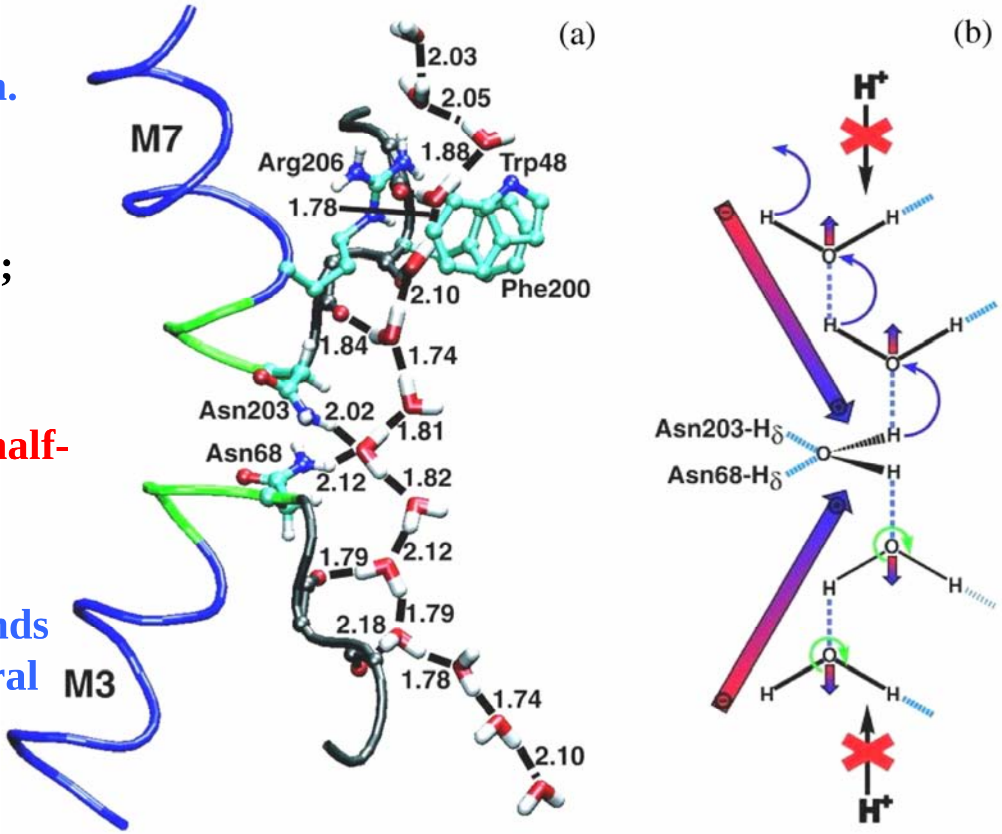
6.4 Anticooperative water-without-proton transmission in aquaporin channels (continued)

Aquaporin channels has been extensively studied by molecular-dynamics simulation.

Figure (a) shows a snapshot from the simulation of *E. Coli* aqua-gliceroporin (Tajkhorshid *et al.*, Science 2002, 296:525; only two α-chains and the water chain are shown).

The snapshot reveals the two antidromic half-chains of H-bonded waters precluding the transmission of the proton (b).

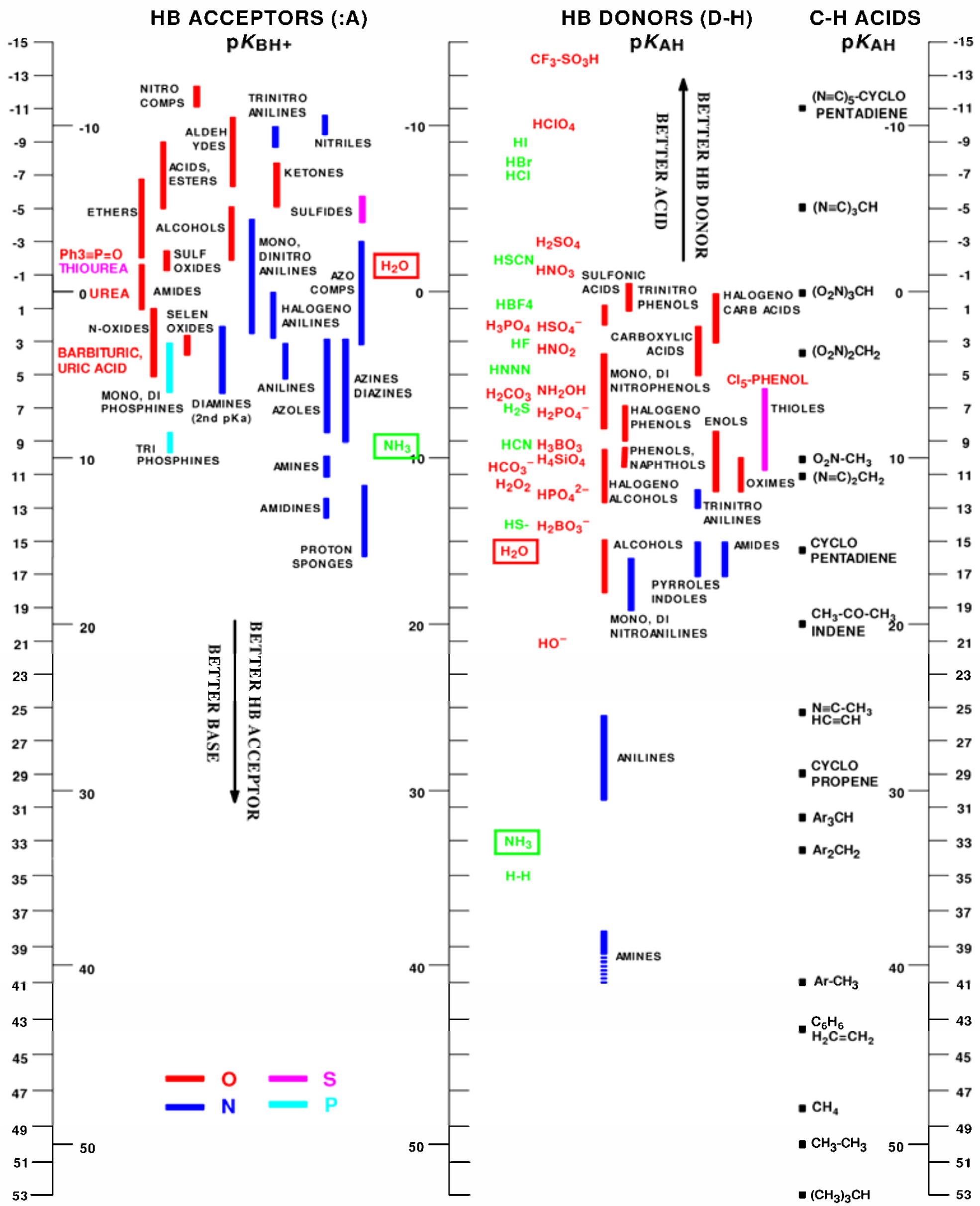
Water rotation is caused by the two H-bonds donated by Asn68 and Asn203 to the central water molecule.



"This water channels in cell membranes movie was made by Drs. Emad Tajkhorshid and Klaus Schulten using VMD and is owned by the Theoretical and Computational Biophysics Group, NIH Resource for Macromolecular Modeling and Bioinformatics, at the Beckman Institute, University of Illinois at Urbana-Champaign."

END of LECTURE 5

Note 1. Predicting H-bond strengths by the pKa Slide Rule



The pK_a Slide Rule

is a **graphical tool for predicting H-bond strengths from the ΔpK_a differences:**

ΔpK_a = pK_{AH}(donor) – pK_{BH+}(acceptor)

- **It contains, in the form of a bar chart, the pK_a values of all the most common classes of D–H donors (A–H acids; on the right) and A: acceptors (B bases; on the left)**
- **different colors indicate the atoms involved**

• • • • •

Strong (±)CAHBs occur when an acid and a base lie on a same horizontal line.

ΔpK_a>> 0: D–H····A, weak & neutral

ΔpK_a ≈ 0: D···H···A, strong & centered

ΔpK_a << 0: $^-$D····~~H~~–A$^+$, weak & charged

Strong (–)CAHBs occur when two acids (on the right) lie on a same horizontal line,

Strong (+)CAHBs when two bases (on the left) lie on a same horizontal line.