

## Protons and Proton Donors

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*For alternative meanings see [proton \(disambiguation\)](#).*

In [physics](#), the **proton** (Greek *proton* = first) is a [subatomic particle](#) with an [electric charge](#) of one positive [fundamental unit](#) ( $1.602 \times 10^{-19}$  [coulomb](#)), a diameter of about  $1.5 \times 10^{-15}$  [m](#), and a mass of  $938.3 \text{ MeV}/c^2$  ( $1.6726 \times 10^{-27}$  [kg](#)), or about 1836 times the mass of an [electron](#). The proton is observed to be [stable](#), with a lower limit on its [half-life](#) of about  $10^{35}$  years, although some theories predict that the [proton may decay](#). The proton has a [density](#) of about  $2.31 \times 10^{17}$  [kg m<sup>-3</sup>](#).

Protons are [spin-1/2 fermions](#) and are composed of three [quarks](#), making them [baryons](#). The two [up quarks](#) and one [down quark](#) of the proton are also held together by the [strong nuclear force](#), mediated by [gluons](#). Protons may be transmuted into [neutrons](#) by [inverse beta decay](#) (that is, by capturing an [electron](#)); since neutrons are heavier than protons, this process does not occur spontaneously but only when energy is supplied. The proton's [antimatter](#) equivalent is the [antiproton](#), which has the *same* magnitude charge as the proton but the opposite sign.

Protons and neutrons are both [nucleons](#), which may be bound by the [nuclear force](#) into [atomic nuclei](#). The most common isotope of the [hydrogen atom](#) is a single proton. The nuclei of other atoms are composed of various numbers of protons and neutrons. The number of protons in the nucleus determines the chemical properties of the atom and which [chemical element](#) it is.

In [chemistry](#) and [biochemistry](#), the proton is thought of as the [hydrogen ion](#), denoted  $\text{H}^+$ . In this context, a proton donor is an [acid](#) and a proton acceptor a [base](#) (see [acid-base reaction theories](#)).

Proton	
Classification	
<a href="#">Subatomic particle</a>	
<a href="#">Fermion</a>	
<a href="#">Hadron</a>	
<a href="#">Baryon</a>	
<a href="#">Nucleon</a>	
<i>Proton</i>	
Properties	
Mass:	$1.672\,621\,71(29) \times 10^{-27}$ <a href="#">kg</a> $938.272\,029(80)$ <a href="#">MeV/c<sup>2</sup></a>
<a href="#">Electric Charge</a> :	$1.602\,176\,53(14) \times 10^{-19}$ <a href="#">C</a>
Diameter:	about $1.5 \times 10^{-15}$ <a href="#">m</a>
Spin:	$\frac{1}{2}$
<a href="#">Quark Composition</a> :	1 down, 2 up
	

proton donor:

The donor of a proton in an acid-base reaction; that is, a Bronsted-Lowry acid

### pH

pH is a measure of effective concentration of hydrogen ions in a solution. It is approximately related to the molarity of  $H^+$  by  $pH = -\log [H^+]$

ORP stands for Oxidation-Reduction Potential. In practical terms, it is a measurement to oxidize contaminants. ORP is the only practical method we have to electronically monitor sanitizer effectiveness. When chemists first used the term in the late 18th Century, the word "oxidation" meant, "to combine with oxygen."

We now know that oxidation involves an exchange of electrons between two atoms. The atom that loses an electron in the process is said to be "oxidized." The one that gains an electron is said to be "reduced." In picking up that extra electron, it loses the electrical energy that makes it "hungry" for more electrons.

We also know that matter can be changed, but not destroyed. You can alter its structure, and can increase or decrease the amount of energy it contains - but you can't eliminate the basic building blocks that make things what they are.

Chemicals like chlorine, bromine, and ozone are all oxidizers. It is their ability to oxidize - to "steal" electrons from other substances - that makes them good water sanitizers, because in altering the chemical makeup of unwanted plants and animals, they kill them. Then they "burn up" the remains, leaving a few harmless chemicals as the by-product.

Of course, in the process of oxidizing, all of these oxidizers are reduced - so they lose their ability to further oxidize things. They may combine with other substances in the water, or their electrical charge may simply be "used up." To make sure that the chemical process continues to the very end, you must have a high enough concentration of oxidizer in the water to do the whole job.

But how much is "enough?" That's where the term potential comes into play.

"Potential" is a word that refers to ability rather than action. We hear it all the time in sports. ("That rookie has a lot of potential - he hasn't done anything yet, but we know that he has the ability to produce.")

Potential energy is energy that is stored and ready to be put to work. It's not actually working, but we know that the energy is there if and when we need it. Another word for potential might be pressure. Blow up a balloon, and there is air pressure inside. As long as we keep the end tightly closed, the pressure remains as potential energy. Release the end, and the air inside rushes out, changing from potential (possible) energy to kinetic (in motion) energy.

In electrical terms, potential energy is measured in volts. Actual energy (current flow) is measured in amps. When you put a voltmeter across the leads of a battery, the reading you get is the difference in electrical pressure - the potential - between the two poles. This pressure represents the excess electrons present at one pole of the battery (caused, incidentally, by a chemical reaction within the battery) ready to flow to the opposite pole.

When we use the term potential in describing ORP, we are actually talking about electrical potential or voltage. We are reading the very tiny voltage generated when a metal is placed in water in the presence of oxidizing and reducing agents. These voltages give us an indication of the ability of the oxidizers in the water to keep it free from contaminants.

If we had a body of water in which the concentration of oxidizers (or oxidants as chemists are apt to say) exactly equaled the concentration of reducers (reductants), then the amount of potential generated at the measuring electrode would be exactly zero. As you might guess, the water would be in pretty sad shape, because if any additional contaminants were introduced into the water, there would be no oxidizer to handle it.

As we add oxidizer to the water, it "steals" electrons from the surface of the platinum measuring electrode. To make things a little more confusing, we need to point out that electrons are negatively charged particles. When we remove these negatively charged things from this electrode, the electrode becomes more and more positively charged. As we continue to add oxidizer to the water, the electrode generates a higher and higher positive voltage.

In 1972, the World Health Organization adopted an ORP standard for drinking water disinfection of 650 millivolts. That is, the WHO stated that when the oxidation-reduction potential in a body of water measures 650/1000 (about 2/3) of a volt, the sanitizer in the water is active enough to destroy harmful organisms almost instantaneously.

In Germany, which has about the strictest water quality standards in the world, an ORP level of 750 millivolts has been established as the minimum standard for public pools (1982) and spas (1984).

ORP devices can be used to measure sanitizer effectiveness and to control ozone generators,

## **Hydrate2O**

### Understanding pH and ORP measurements

ORP stands for Oxidation – Reduction Potential

It measures the potential for a liquid solution to be an Oxidizer or a Reducer.

Oxidizers – Accept electrons or strip electrons from another compound. This is what free radicals do in the body. They strip electrons from the cell membrane walls or from the DNA of the nucleus and damage the cell. Free radicals are opposed or neutralized by anti-oxidants. The mitochondria of the cells, where energy is produced, generate free radicals in the form of highly charged oxygen (singlet oxygen, O<sup>2-</sup> and OH<sup>-</sup>) and metabolic waste. Very powerful anti-oxidants, like Coenzyme Q10, Alpha-Lipoic Acid and Lycopene surround the mitochondria to prevent the escape of the free radicals.

The metabolic waste is generally a weak acid, ie lactic acid. Liquid solutions with a positive ORP are oxidizers and have an acidic pH. Water with dissolved oxygen will have a positive ORP and be a weak oxidizer. Iron will rust in well water, but bacteria will grow. Ozone and Chlorine are strong oxidizers and are powerful disinfectants when added to water. The ORP will be very high and bacteria die. ORP is used in many industries to measure water quality. A water with a high ORP will also be a low pH or acidic water. Acid solutions are proton donors (H<sup>+</sup>). A solution of HCL, hydrochloric acid, is an ionic mixture of H<sup>+</sup> and CL<sup>-</sup>. The pH will be very low, acidic, depending on the concentration of the HCL added to the water and the ORP will be high. Stomach acid for example is used to destroy incoming bacteria. It is a strong oxidizer and disinfectant. It also starts the breakdown of proteins. ORP and pH are used together to control many chemical reactions

Reducers – Donate electrons or give up electrons to other compounds. Anti-oxidants give up electrons to the free radicals to neutralize them. A liquid solution that has a negative ORP will be a reducer. It will give up electrons. Anti-oxidants are unique in that they can give up an electron without becoming a free radical in search of an electron. Negative ORP readings are also associated with a high pH value or alkaline. They can donate electrons or accept protons. By accepting protons alkaline solutions neutralize acids. Pure water is a neutral pH of 7 and a 0 ORP. Additives move both the ORP and the pH. Certain minerals can raise the ORP and other minerals lower the ORP. Tap water can be through an ionizer to create two separate streams. One stream will have a positive ORP and low pH in response to the acidic minerals it contains and the other will have a negative ORP and high pH and be called alkaline water. The degree of separation depends on the quality of the machine. Alkaline water is thought to be healthy to drink to help neutralize the acidic waste being generated in the body. The acidic water is thought to be good to wash your hands with because of its antiseptic properties. It is also recommended to water your plants with the acid water to improve soil conditions. Hence there is a thought that the alkaline water with its high pH and low ORP is the standard for judging healthy water. That is a mistake.

An alkaline water used long term is going to slowly neutralize the stomach acid and change the pH of the intestinal tract. This will lead to long term problems with protein and fat metabolism. Both are highly pH dependent. There will also be a slow accumulation of pathogenic bacteria in the gut which can produce ulcers and inflammation of the gut, such as Chron's disease. The upper part of the GI tract is designed to operate at a fairly acidic pH of 5.6. A high pH water may be good for someone who is highly toxic and producing a large amount of metabolic waste. But it is a short term solution. Also the alkaline waters have not been demonstrated to be hydrating to the cells. They may add some minerals to buffer the blood pH, but that does not mean the water tranverses through the aquaporin channels to hydrate the cells.

What you really want to do is eliminate the production of the metabolic waste. Since metabolic waste is generated in the Krebs cycle of the mtichondria, it is critically important to realize how

the body produces energy through the conversion of ADP to ATP. This is described in the paper Applied BioLogics found on The Stowe Foundation website, [www.thestowefoundation.org](http://www.thestowefoundation.org). The mitochondria are the organelles contained within each cell of the body that produce energy. The mitochondria use the enzymes of the Krebs cycle to convert blood glucose into energy. These enzymes must be contained in cellular water to operate efficiently. The interior of the mitochondria is the deepest spot that water must penetrate. Any water that can not deliver superior cellular hydration will not affect the hydration of the mitochondria. Dehydrated mitochondria will short circuit the electron transfer capacity of the mitochondrial enzymes. Energy production is inefficient and the cells generate lots of metabolic waste.

In other activities, such as intense exercise, oxygen to the muscles is in short supply and the muscles will begin to use anerobic pathways to keep producing energy for as long as possible. These enzymes require proton donors and the body will produce lactic acid as the source of protons. When the exercise ceases the lactic acid must be swept from the cells, which also requires cellular hydration. The key and most fundamental property of water is very simple. Does it hydrate the cells or not? Hydrate2O has proven to have the right structure and physical properties to hydrate the cells. It also has the correct ORP and pH to support and not degrade the intestinal tract. That is why you never get the intestinal bloating when you drink Hydrate2O. Also Hydrate2O can serve as a proton donor deep within the mitochondria to keep energy production efficient and hence limit the production of metabolic free radicals. Hydration is the key and the ability of being a proton donor is a very special feature.

The ORP measurements of Hydrate2O show that our ozone process has disinfected the water and dissolved extra oxygen into the water. It also shows that Hydrate2O can be a proton donor. By hydrating the cells all the way to the level of the mitochondria with a proton donating water, Hydrate2O has established a new category of water. It should not be compared to alkaline water that has limited benefits to the body. Anti-oxidant waters limit the damage of energy production, but Hydrate2O improves the efficiency of energy production and therefore allows the body's normal anti-oxidant systems to be sufficient. In fact, our sports energy drink will enhance the proton donor capacity of the water and will write a new chapter in sports physiology.

How The Mitochondria Work

Condensed from the internet

## PROTON MOTIVE FORCE

The **chemiosmotic theory** (*def*) explains the functioning of electron transport chains. According to this theory, the transfer of electrons down an electron transport system through a series of oxidation-reduction reactions (*def*) releases energy ([see Fig. 1](#)). This energy allows certain carriers in the chain to transport hydrogen ions ( $H^+$  or protons) across a membrane.

[Animation illustrating energy release during oxidation-reduction reactions.](#)

Depending on the type of cell, the electron transport chain may be found in the cytoplasmic membrane or the inner membrane of mitochondria.

- In [prokaryotic cells](#), the protons are transported from the cytoplasm of the bacterium across the cytoplasmic membrane to the periplasmic space located between the cytoplasmic membrane and the cell wall .
- In [eukaryotic cells](#), protons are transported from the matrix of the mitochondria across the inner mitochondrial membrane to the intermembrane space located between the inner and outer mitochondrial membranes ([see Fig. 4](#)).

As the hydrogen ions accumulate on one side of a membrane, the concentration of hydrogen ions creates an **electrochemical gradient or potential difference (voltage) across the membrane**. (The fluid on the side of the membrane where the protons accumulate acquires a positive charge; the fluid on the opposite side of the membrane is left with a negative charge.) The energized state of the membrane as a result of this charge separation is called **proton motive force (def) or PMF**.

This proton motive force provides the energy necessary for enzymes called **ATP synthases** ([see Fig. 5](#)), also located in the membranes mentioned above, to catalyze the **synthesis of ATP** from ADP and phosphate. This generation of ATP occurs as the protons cross the membrane through the ATP synthase complexes and re-enter either the bacterial cytoplasm ([see Fig. 2](#)) or the matrix of the mitochondria. As the protons move down the concentration gradient through the ATP synthase, the **energy released causes the rotor and rod of the ATP synthase to rotate**. The mechanical energy from this rotation is converted into chemical energy as phosphate is added to ADP from ATP.

[Animation illustrating ATP synthase generating ATP.](#)

Proton motive force is also used to transport substances across membranes during active transport and to rotate bacterial flagella.

At the end of the electron transport chain involved in **aerobic respiration**, the last electron carrier in the membrane transfers 2 electrons to half an **oxygen** molecule (an oxygen atom) that simultaneously combines with 2 protons from the surrounding medium to produce **water** as an end product ([see Fig. 3](#)).

[Animation illustrating the development of proton motive force as a result of chemiosmosis.](#)

[Animation illustrating the formation of ATP from proton motive force and ATP synthase.](#)

[Animation illustrating ATP production by chemiosmosis during aerobic respiration.](#)

The cell energy production circuit is known as the Krebs cycle and is located inside the mitochondria of each cell. A mitochondrion is the organelle that produces energy for the cell and each cell has multiple energy production centers. It is the energy production cycle that produces free radicals such as singlet oxygen that can damage the cell. In order to prevent cell damage, the mitochondria are surrounded with anti-oxidants and specific enzymes, such as super oxide dismutase (SOD) and glutathione, to neutralize and control damaging free radicals. Our life force is maintained by the production of energy, so we are always producing free radicals. Therefore, it is critical that we maintain a high degree of quality control over the energy production cycle. The body breaks down without proper maintenance on the engine.

Peter Mitchell, a British chemist, won the 1978 Nobel Prize for chemistry for helping to clarify how ADP (adenosine diphosphate) is converted into the energy carrying compound ATP (adenosine triphosphate) in the mitochondria of living cells. ATP is made within the mitochondria by adding a phosphate group to ADP in a process known as oxidative phosphorylation. Mitchell was able to determine how the different enzymes involved in the conversion of ADP to ATP are distributed within the membranes that partition the interior of the mitochondrion. He showed how these enzymes arrangement facilitates their use of hydrogen ions (protons,  $H^+$ ) as an energy source in the conversion of ADP to ATP. Later scientists have described this phenomenon as the proton motive force and the proton wave effect. The Krebs cycle requires a source of protons ( $H^+$ ) from proton donors.

The most common proton donor studied in the Krebs cycle, also referred to as the citric acid cycle, is nicotinamide adenine dinucleotide (NADH). NADH is a coenzyme made from vitamin B2 or niacin. It is present in all living cells. As a coenzyme, NADH serves to make the enzymes of the Krebs cycle work. The Krebs cycle is often described more in terms of an electron transport mechanism in which enzymes catalyze sequential oxidation reduction reactions. The fuel for the Krebs cycle is blood glucose and the agent for oxidation is oxygen. However, hydrogen protons ( $H^+$ ) are required for the reduction reactions. Therefore, the electron transport

required to generate energy can only occur when the sequential oxidation reduction reactions are coupled together. The coupling mechanism is provided by the proton flux and this requires the presence of proton donors.

Very advanced work by Markus Meuwly and published in the Faraday Discussions suggests that the diffusion of water (H<sub>2</sub>O) into the active site of an electron coupled proton transfer is an attractive alternative to direct proton transfer from the protein matrix of ferredoxins. The ferredoxins are proteins that contain one or more iron-sulphur clusters, which makes such proteins eminent in electron transfer reactions. Ferredoxins are a major component of the Krebs cycle. Meuwly's work suggests that the water molecule could serve as a possible proton donor.

Meuwly's work demonstrates why chronic illness is always associated with dehydration at the cellular level. The mitochondria are being deprived of a source of proton donors, cellular H<sub>2</sub>O, which is also biomolecular water or structured water. The water in our cells is an active participant in the chemical reactions that occur in our mitochondria. The mitochondria are the original hydrogen fuel cell.

The body uses a complex system of catalysts (enzymes and coenzymes) within the mitochondria to achieve energy efficiency. A catalyst is a compound that can lower the activation energy of a given chemical reaction. In the human body, catalysts are known as enzymes and the coenzymes that make the enzymes work. We know that many coenzymes are derived from the B-vitamins and hence diet plays critical role in health.

A second major factor in the proper function of the enzymes are the metals (trace minerals in nutritional terms) attached to the amino acid sequences that form the basic protein structure of the enzyme. It is the trace minerals that easily transfer electrons from one compound to another. This is the heart and sole of energy production, the transfer of electrons. A fundamental concept in the attachment of a trace mineral or metal to a protein structure is the binding energy of the metal. How easily does the metal or trace mineral attach to the enzyme? Basically, metals are competitive with each other. This has major implications in health.

It is straight forward science that a metal, like cadmium, found in the paper of cigarettes and inhaled with every puff or breathed in through second hand smoke, can displace the biologically active metals of zinc, selenium and magnesium (the trace minerals) from the biologically active enzymes. Cadmium has a stronger binding coefficient for the amino acid structure of the enzyme than the trace minerals. When cadmium or mercury or any other heavy metal displaces a trace mineral like zinc or selenium from the enzyme it does not function properly as a catalyst. Electrons flow undirected through the cellular matrix and free radical pathology can spin out of control. You can not take enough anti-oxidants to keep up with the free radical production and hence inflammation becomes a permanent part of your life. The energy balance of the body is disrupted. In the oil refining industry they would say the catalyst has become contaminated or

poisoned. In that event, the refinery catalyst is replaced with a fresh batch. This is not so easily accomplished in the body.

In the human body, the proper energy balance can only be restored by decontaminating or detoxifying the body. That is, remove the catalytic poisons (like cadmium, lead, mercury, arsenic, aluminum, nickel) that are attached to the enzymes and replenish them with the proper energetic metals like selenium and zinc. This is the essence of chelation therapy and trace mineral replacement. Remove the toxic heavy metals and restore the beneficial trace minerals. Then the mitochondria can function and our life force restored. Through the concepts of Applied BioLogics, we understand the importance and the science of energy medicine. Making sure the body operates at the right energy levels is pure science and engineering. When enzymes start to fail on a systemic basis, then it is time to look at enzyme therapy, with enzyme supplements to compensate for the dysfunctional pathways. It is also time to look at how to detoxify the body. The body will only be restored to true health when the enzyme poisons have been removed and proper controls put on free radical pathology.

In fact, there are encouraging signs that the concepts of energy medicine are starting to permeate the medical field. Work performed at the Mayo Clinic by Dzeja and Terzic states; “at the level of the mitochondria and the nucleus of the cell, precise coupling of spatially separated intracellular ATP-producing and ATP-consuming processes is fundamental to the bioenergetics of living organisms, ensuring a fail-safe operation of the energetic system over a broad range of cellular functional activities. Mechanisms responsible for communication between spatially separated intracellular ATP consumption and ATP production processes, and their precise coupling over a broad range of cellular functional activity has remained a long-standing enigma. Distribution of cellular energy could be accomplished by altering phosphotransfer enzyme isoform composition and their intracellular localization. In this regard, derangement in cellular energy flow and distribution has been implicated in cardiovascular and neurodegenerative diseases, as well as in determining uncontrolled growth and metastatic potential of tumor cells”.

The cliff note version of these statements is that enzymes contained in the mitochondria, the cytoplasm and the nucleus of the cell are responsible for the energy production, the energy transfer and the energy utilization within the cell. The enzymes must be located a proper distance from each other in order to achieve a balanced flow of energy. When energy flow is disturbed then cells do not function properly and major problems show up, such as heart disease, diabetes, dementia and cancer.

Functional medicine must look at the energy status of the body and this requires a very close examination of the enzymatic pathways of the body. The primary determinant of the spatial alignment of enzymes is cell hydration. Cellular water determines cell volume and whether the cell is swollen or dehydrated. Too much water and the enzymes will be too far apart. Too little cellular hydration and the enzymes will interfere with each other. Cellular hydration plays two

critical roles in cell health, the spatial separation of the enzymes and the potential to be a proton donor.

Enzymes that are contaminated with toxins are not going to work. Make no mistake, America is loaded with toxins. The heavy metal toxins are quite prevalent. Mercury is found in the fish we eat and the silver fillings we put in our mouth, cadmium is found in cigarette smoke, arsenic throughout many of the water supplies due to industrial pollution and lead from paints and fuels. Aluminum and nickel are being monitored as contributing factors to Alzheimers and Parkinson's disease. We even use barium in medical diagnostic tests. Exposure to heavy metals is easy to come by and the heavy metals have an affinity to attach themselves to the enzymes. The body will accumulate a toxic load over time. It shows up in our bodies as accelerated aging and chronic illness. Tissue samples taken from breast cancer patients often show 30,000 times the level of mercury as thought safe.

The FDA and EPA recently released warnings to pregnant mothers to avoid mercury contaminated fish, which included tuna and swordfish. The reason given was to avoid damage to the developing fetus, in particular, to avoid fetal brain damage. The mother should be well advised that mercury is not selective to the fetus. It will harm her as well as the fetus.

Mercury is even used in laboratory experiments as the toxin most easily applied to disrupt the flow of water into human cells through the aquaporin channels. The aquaporin channels are essentially enzymatic pathways that transport water into and out of the cells and cellular water into and out of the organelles of the cells like the mitochondria.

The aquaporin channels depend on the proper electrical charge on the enzymes to function. Mercury or any heavy metal can easily disrupt the electrical field of the aquaporin channel. Dehydration at the cellular level is common among all chronic illness. This can easily be the result of toxins shutting off the aquaporin channels. Clinical studies show that hydration affects human performance at every level of the body. Toxins interfere with hydration and the enzymatic performance in the cells, the mitochondria and the cell membrane walls. Heavy metals are very nasty enzyme poisons and must be considered a cofactor in every chronic illness. But heavy metals are not the only toxins that can affect enzyme performance.

Most of the chemical toxins we are exposed to in our daily lives, such as herbicides, insecticides and pesticides are chlorine or fluorine based. Agent Orange is probably the best known herbicide and it is extremely toxic to the human body. Fluorine and chlorine are chemically known as halogens and are extremely reactive substances. We are advised not to swallow our fluoridated toothpaste and to not use it with infants. Exposure to halogens also comes from flame retardant chemicals found on our carpets and upholstery plus baby clothes and cribs. These toxins can also disable enzymatic activity and membrane permeability which leads to

uncontrolled free radical pathology and the accumulation of metabolic waste. Toxins are the silent killers.

Markus Meuwly:

### Electron Coupled Proton Transfer in Ferredoxins

Ferredoxins are proteins that contain one or several iron-sulphur clusters. This makes such proteins eminent in electron transfer reactions. FdI is a particularly intensely investigated iron-sulphur protein for which also mutants have been crystallized and spectroscopically characterized. This allows to compare theoretical investigations with a variety of experimental data and validate the approach taken.

Because Fd I (PDB code 7FDR) contains a [3Fe-4S] and a [4Fe-4S] cluster, first parameters for describing the electrostatics and the bond-, angle-, and dihedral-terms in the molecular dynamics force field have to be derived. This is done using *ab initio* calculations. Density functional theory has shown to be very suitable for such investigations. We used UB3LYP with the 6-31G\*\* basis set to calculate optimized structures of the isolated [3Fe-4S]<sup>(0/+)</sup> and the biologically more relevant [3Fe-4S]<sup>(0/+)</sup>(SCH<sub>3</sub>)<sub>3</sub> cluster which is closer to the situation in the protein. Charges are calculated using standard Mulliken, the ESP and NBO method. All three charge sets are used in the molecular dynamics calculations.

Analysis of the experimental results suggested that the protonation occurs directly from the protein matrix to the FeS cluster. However, in our MD simulations, we observed a number of other candidate-hydrogen atoms that could possibly serve as proton donors. In particular, the suggested proton at Asp15 is generally too far away from the FeS cluster to serve as an ideal candidate. The other protons from surrounding side chains are usually strongly bound. A possible reaction mechanism involves **the additional protonation of the protein side chain from solvent water which would make the NH of Asp15 less acidic. However, an attractive alternative to direct proton transfer from the protein matrix is the diffusion of water into the active site. We have shown that such a water molecule is dynamically stable and indeed could serve as a possible proton donor.**[1] Also, differences between a fast (native protein) and slow (D15E mutant) variant of the protein were observed in the MD simulations that could account for the observed slowdown of an order or magnitude upon mutating Asp15-->Glu15.[2,3]

[1] M. Meuwly and M. Karplus, Farad. Disc. Royal. Soc. 124, 297 (2003)

[2] K. Chen, J. Hirst, R. Camba, C. A. Bonagura, C. D. Stout, B. K. Burgess and F. A. Armstrong, Nature, 405, 814-817 (2000)

[3] M. Meuwly and M. Karplus, Biophys. J., in print (2004)

## ***Is Hydrate20 a proton donor***

### ***Uses of nuclear magnetic resonance***

The most obvious use of nuclear magnetic resonance is in [magnetic resonance imaging](#) for medical diagnosis, however, it is also widely used in chemical studies, notably in [NMR spectroscopy](#) such as [proton NMR](#) and [carbon-13 NMR](#).

These studies are possible because nuclei are surrounded by orbiting electrons, which are also spinning charged particles such as [magnets](#) and, so, will partially shield the nuclei. The amount of shielding depends on the exact local environment. For example, a hydrogen bonded to an [oxygen](#) will be shielded differently than a hydrogen bonded to a carbon atom. In addition, two hydrogen nuclei can interact via a process known as [spin-spin coupling](#), if they are on the same molecule, which will split the lines of the spectra in a recognisable way.

By studying the peaks of nuclear magnetic resonance spectra, skilled chemists can determine the structure of many compounds. It can be a very selective technique, distinguishing among many atoms within a molecule or collection of molecules of the same type but which differ only in terms of their local chemical environment.

By studying  $T_2^*$  information a chemist can determine the identity of a compound by comparing the observed nuclear precession frequencies to known frequencies. Further structural data can be elucidated by observing *spin-spin coupling*, a process by which the precession frequency of a nucleus can be influenced by the magnetization transfer from nearby nuclei.

$T_2$  information can give information about dynamics and molecular motion.

The dynamics and molecular motion of the hydrogen proton can be directly correlated to the physical properties of the molecule.

## ⊖ ANALYTICAL TEST RESULTS ⊕

### Hydration Specialties™ Structured Water Hydrate2O

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#### Introduction

The purpose of the testing program was to characterize the unique properties of **Hydration Specialties** Structured Water (HS S W) in contrast with other waters and to explain the potential effects of these characteristics upon human physiology. *There is no standard test to measure the molecular state of water.*

#### Methods

**Hydration Specialties** Structured Water, tap, electrolyzed, chemically additive, mineral and other bottled waters were analyzed by accredited laboratories for the measurement of such physical properties as conductivity, boiling point, viscosity, surface tension, and Nuclear Magnetic Resonance signature.

#### Results

Laboratory analyses indicate that Hydration Specialties Structured Water has a unique combination of consistent boiling point, lower viscosity, and excellent conductivity signature.

NMR signature demonstrated the HS SW has a lower vibrational frequency measured in Hertz (Hz).

These results all indicate a 'wetter', or 'thinner' water, exhibiting the physical characteristics of smaller clusters of water molecules.

#### Conclusions

Theoretically, these properties enhance rapid cellular absorption, thereby inducing true hydration and creating an effective biological delivery agent.

Smaller clusters of water molecules enhance transport across cell membranes, promoting intracellular hydration, and removal of metabolites.

## Testing Sites and Measurements

Surface tension, viscosity & conductivity:

**Truesdail Laboratories, Inc., Tustin, CA**

Method: Truesdail Laboratories analyzed these waters in accordance with ASTM Method D-445 for viscosity and ASTM Method D-1331 for surface tension, and ASTM Method B-2510 for conductivity.

Boiling point analysis:

**West Coast Analytical Service, Inc., Santa Fe Springs, CA**

Method: WCAS analyzed these waters in accordance with USP 24 Method 721 for boiling point.

Nuclear Magnetic Resonance spectrometry:

**Acorn NMR Laboratory, Livermore, CA**

Method: Oxygen Isotope 17 method

All laboratories implemented industrial quality assurance and quality control standards to obtain their results. **In all instances only sample number identified test samples, so the test operator had no knowledge of sample identity.**

The results and significance of each test are presented below.

## The Importance of Water to the Human Body

The basic living unit of the body is the cell. These cells are all 'bathed' inside and outside with water. The body is 70-75 % water by weight. The water inside the cell is called **intracellular water**, whilst that outside is referred to as **extra cellular water**.

The water molecule may seem a simple inorganic molecule, but it plays a critical part in life's functions. Water is an active participant in ALL biochemical and metabolic reactions within the cells. Without an adequate supply of water, these reactions are compromised or simply do not take place.

The hydrogen and oxygen atoms of water are joined by **polarized covalent bonds**. This polarization allows water molecules to form hydrogen bonds with other water and polarized molecules. It is the prevalence of this ability that causes water to become bound in large macromolecules.

These large arrays of bound water are highly restricted from passing through the cellular membrane. Hydration Specialties' process structures water by **breaking a portion of these hydrogen bonds**. This results in **smaller clusters of water molecules with reduced surface tension or cohesion**. This lower surface tension enables HS S W to saturate the intracellular area more rapidly and more efficiently.

### **Cellular Absorption and Detoxification**

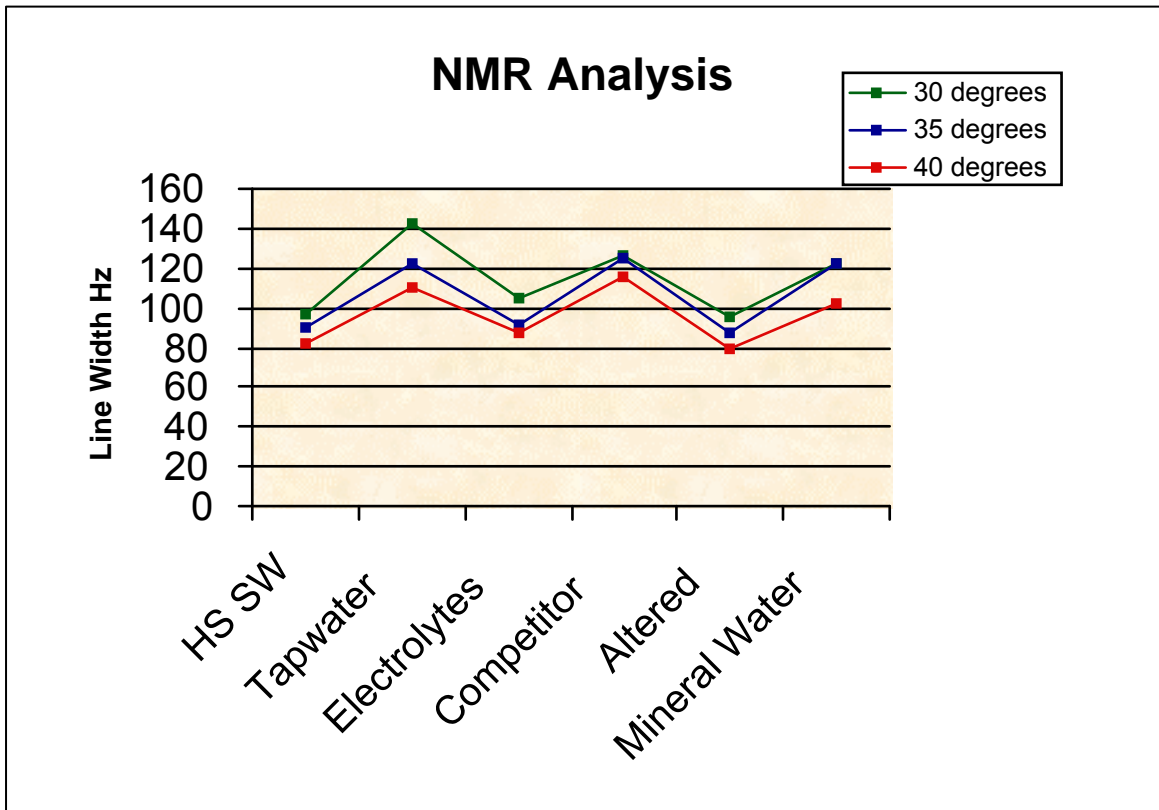
The water that we drink differs substantially from the bound water that surrounds the tissue and cells in the body. It is necessary for the body to reduce the surface tension of water we consume in order for the nutrients to pass through the cell membrane and so metabolites (waste products) can be excreted from the cell. Water molecule cluster size is a relative indication of surface tension. Smaller water clusters have relatively less cohesion as a result of a fewer number of hydrogen bonds between the individual water molecules. The surface tension is decreased because the water clusters are smaller.

### ***NMR***

Hydration Specialties Structured Water has the unique physical properties associated with 'smaller water clusters' as measured by nuclear magnetic resonance.

NMR is a spectroscopic technique, which uses **electromagnetic radiation and magnetic fields to determine the structure of molecules**. RF radiation is used to stimulate the nuclei present within the molecule and from the information obtained by this process the location of the hydrogen and carbon atoms can be accurately determined. The principle of NMR is based upon the spin of atomic nuclei in an external magnetic field.

NMR Test results for Hydration Specialties Structured Water is displayed below:



### NMR Test Results

Acorn NMR Laboratories in Livermore, CA

NMR studies were performed on:

- HS SW
- Electrolyzed water
- Chemically altered water (using additives)
- Mineral water
- Tap Water
- Miscellaneous bottled water

The NMR signal is displayed graphically by a single peak or line, measured in hertz (Hz), with the width of the peak being the determinant factor. In a solution, the larger the molecular clusters (i.e. the more hydrogen bonds), the rotational diffusion, or spin, is slower. This results in an NMR signature with

broader peak or line width. **The smaller the clusters of molecules then the narrower the peak or line width.**

Therefore, the significance of the line width is as a determinant of relative size of molecular clusters in the respective samples. As the graph so vividly illustrates, the peak or line width for HS SW suggest reduced cluster sizes relative to all other samples save for the two with chemical additives. The additives in these two sample waters were primarily metasilicates, known for their ability to act as a catalyst to chemically enforce the reduction in hydrogen bonding, thereby inducing the property of smaller aggregate clusters.

**HS SW uses neither chemicals nor additives of any kind to achieve their unique physical properties of smaller water clusters as measured by NMR 170.** The smaller clusters of Hydration Specialties water are able to saturate the cell more rapidly and thereby enhance the absorption and detoxification of the cells' fluid volume.

## ***CONDUCTIVITY***

Truesdail Laboratories, Irvine CA

Conductivity is the **ability of an electrical current to pass through a medium.** Electrical conductivity in water is a measure of the ion facilitated electron flow through the water. The concentration of these ions is the factor that dictates the level of conductivity.

Every function occurring in the body requires communication from one cell to another. These messages are conducted by the passage of electrons through our extra-cellular fluids. **An optimal level of conductivity in these fluids is essential to life and cellular activity.**

The standard method ASTM 2510-B procedures specify conductivity measurements as criteria for characterizing water. In addition to defining the test protocol, ASTM 2510-B sets performance standards for the conductivity measurement system, as well as validation and calibration requirements for the meter and conductivity.

### **Conductivity Test Results**

***The conductivity of Hydration Specialties Structured Water is as expected from a non-electrolyte fortified water. The Conductivity measurement of 365 ( $\mu\text{mhos/cm}$ ) indicates an excellent level of physiological conductivity.***

## ***BOILING POINT ANALYSIS***

West Coast Analytical Service, Inc. Santa Fe Springs, CA

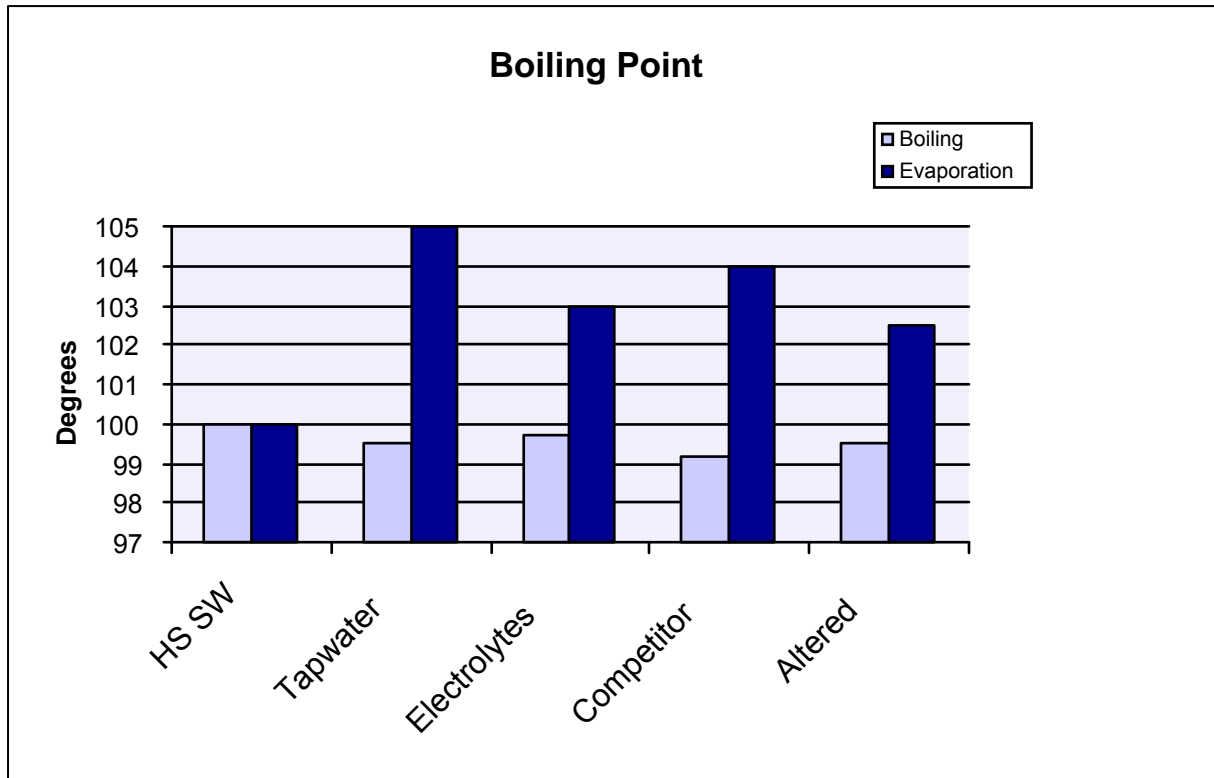
Distillations were carried out on

- HS SW
- Chemically altered water (using additives)
- Electrolyzed water
- Mineral water
- Miscellaneous bottled water

Distillation is a classical method of evaporation through applied heat to determine the range of temperatures within which a liquid distils.

## **Boiling Point Test Results**

The test results, expressed in degrees Centigrade as a distillation range, are graphically displayed below and show a marked difference in the characteristics of these waters.



The lower limit of the range, or the initial boiling point, is the temperature indicated by the thermometer when the first drop of condensate leaves the tip of the condenser. The upper limit of the range is the Dry Point, the temperature at which the last drop of liquid evaporates from the distillation flask.

The wider the range of distillation directly correlates to more substances in the solution. The higher the upper limit, or Dry Point, in the range is indicative that some component(s) is present that is preventing the liquid from complete evaporation.

HS Structured Water was unique in that it began boiling and completely evaporated immediately at the same temperature. **These boiling characteristics indicate an extremely pure substance, with uniformity in the internal composition of materials.** This is intuitively the distillation profile one would expect from a material that has a consistent, uniform structure. **This data strongly suggests the presence of the smaller, well ordered, cluster of water molecules.**

These test results are highly significant and indicates that the HS SW exhibits substantially different behavior from the other waters tested.

The data illustrates that the Hydration Specialties structured water possess characteristics and physical properties substantially different relative to the other waters. **The boiling point profile for HS SW strongly**

suggests the presence of a consistent cluster size. This also suggests that the structured water process has changed the compositional make-up of the molecular structure.

## ***VISCOSITY***

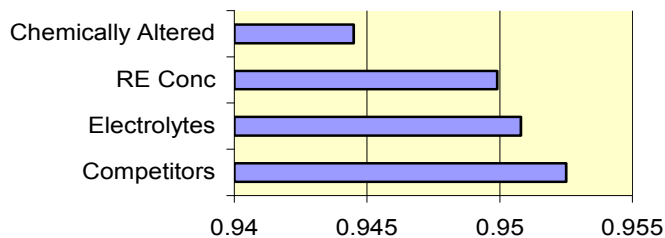
Truesdail Laboratories, Inc. Tustin, CA

Tested in accordance with ASTM Method D-445. In this method the time is measured in seconds for a fixed volume of liquid to flow under gravity through the capillary of a calibrated viscometer under closely controlled temperature. The kinematical viscosity is the product of the time and the calibration constant of the viscometer. The calibration constant of the viscometer is determined by running certified standard solutions of known viscosity.

### **Viscosity Test Result**

The results of the viscosity test are presented graphically below. Again, the results indicate a necessary marked difference in composition between HS SW and the other waters tested. The behavior of the Hydration Specialties Structured water is so significantly varied as to again demonstrate that it has unique physical properties.

Water	Viscosity
Competitors	0.9525
Electrolytes	0.9508
RE Conc	0.9499
Chemically Altered	0.9445



Viscosity is a measure of the resistance to flow in a fluid. In the analysis performed, the higher the number, the greater the viscosity. The more viscous the fluid, the more resistance to flow and hence, the slower the flow.

Water is a means of transportation in the body; a vehicle by which nutrients are carried to the cells throughout the body and waste is carried away. Ideally, the less viscous the water, or the 'thinner' or 'wetter' the water is, the more rapidly it can perform its biological functions. This comparison indicates that HS SW exhibits the characteristics of 'thinner or wetter' water, which is consistent for water with a structure of smaller molecular clusters.

Two other samples also showed reduced viscosity, but both contain chemical additives, which enforce a modified structure onto the solution. HS SW contains no chemicals or additives of any kind.

### **SURFACE TENSION ANALYSIS**

Truesdail Laboratories, Inc. Tustin, CA

The surface tension measurements are run according to ASTM Method D-1331. This method employs the use of a CSC-Du Nouy Tensiometer. The tensiometer uses a fine torsion wire to apply the necessary force to withdraw a platinum-iridium ring from the surface of the liquid under test. The torsion wire is connected to a graduated dial with a vernier scale that permits reading the applied force directly to 0.1 dyne.

**Hydration Specialties Structured Water has been specifically engineered to rapidly restore intracellular fluids to their optimum state by taking advantage of the process Nature uses, the phenomenon of osmosis.** Solutions that have identical osmotic pressure are said to be isotonic solutions. For optimal and rapid replenishment of intracellular fluids, the replenishing fluid must be isotonic with intracellular fluids.

For example, if cells are bathed in a hyper tonic solution, which is a solution having osmotic pressure higher than that of intracellular fluids, the cell will shrivel and because of a net transfer of water out of the cell. The opposite situation occurs when cells are bathed in a solution with an osmotic pressure lower than that of the intracellular fluids. In this case the cells rupture because of the net flow of water into the cell.

As we age, there is a definite predisposition for the composition of body water to shift from intracellular to extra cellular. The ratio of intra to extra water content changes from approximately 1.1 to almost 0.8 between the ages of 20 to 70. The realization is that as we age, we become chronically dehydrated with a decreased ability to re-hydrate. The body then loses its reserve capacity of protein and diminishes its enzyme function. Enzyme functions are optimum only with proper hydration.

The major determinants of intracellular and extra cellular fluid volume homeostasis are separate. **Sodium balance regulates extra cellular fluid volume while sufficient hydration regulates intracellular fluid volume.** This suggests that the loss of intracellular water accompanying the aging process may be minimized or totally negated by an individual's conscious decision to maintain proper hydration before thirst is perceived.

### Surface Tension Analysis Results

Hydration Specialties Structured Water - 67.8 dynes @ 25° C

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