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LIQUID CRYSTALLINE WATER, QUANTUM MOLECULAR MACHINES & THE LIVING STATE

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Abstract: It has been 18 years since the discovery in my laboratory suggesting that living organisms are dynamic liquid crystalline phases in which *all* the molecules in the cells and tissues of the body are aligned and moving coherently together, including especially the water molecules that are intrinsic to the liquid crystalline matrix and essential to the function of macromolecules. In effect, the organism depends on a macroscopic quantum coherence of individual molecular energy machines.

Recent investigations using a range of sophisticated spectroscopic techniques have indeed revealed that the liquid crystalline (hydration) water forms dynamically coherent units with proteins and nucleic acids, and enables the macromolecules to function coherently together.

The same investigations also reveal that ions play their pivotal role in cellular metabolism and signalling through interactions with hydration water. Despite these major advances, the ultimate mystery of life remains tantalizingly beyond our grasp.

Keywords: liquid crystalline organism, quantum coherence, molecular energy machines, hydration water, two-states water, kosmotrope and chaotrope, Law of matching water affinities, microdomains.

THE LIQUID CRYSTALLINE ORGANISM

It's been 18 years since I peered down the polarised light microscope and saw what no one else has seen before: a little fruit fly larva doing a psychedelic dance in all the colours of the rainbow. The polarizing light microscope technician was on leave, so we were left to our own devices. My colleague Michael Lawrence turned all the knobs this way and that until we got the best contrast. We had stumbled on a new configuration that optimised the detection of low level birefringence typical of biomolecules. But that alone would not have enabled us to see the dynamic liquid crystalline display of the little fruit fly larva.

For that to happen, *all* the molecules in the cells and tissues of its body must be aligned *and* moving coherently together. Not just the macromolecules but more importantly, the cell and tissue water must be an intrinsic part of the polarised liquid crystalline phase; the water forming dynamically coherent units with the macromolecules embedded and immersed in it. This water I call liquid crystalline water. The fruit fly larva is not unique, all living cells and organisms display themselves like that.

In the book named after the fruit fly larva [1], *The Rainbow and the Worm, The Physics of Organisms* first published in 1993 and now in its 3rd enlarged 2008 edition, I present theoretical arguments and empirical evidence to support the idea that the organism is coherent to a high degree, even quantum coherent; and liquid crystalline water is an intrinsic part of the quantum molecular machines that power cells and organisms.

A quantum molecular machine transfers and transforms energy quantum mechanically at the molecular level without dissipation. This is exactly what motivates a lot of nanotechnology.

However, nanotechnologists still have much to learn from nature in how liquid crystalline water associated with nanomolecular machines is crucial to their functioning. Prof. Djuro Koruga is a pioneer among nanotechnologists in his multifaceted research on water and nanomaterials, and I thank him for inviting me to contribute to his vision.

I shall describe some recent evidence in support of the view that liquid crystalline water forms dynamically coherent units with enzyme proteins and DNA and then see how that might explain the living state.

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PROTEIN HYDRATION

Organisms have an enormous repertoire of chemical reactions that enable them to transform energy and materials for growth, development, and to do all that's required of being alive. These chemical reactions are catalyzed by specific enzyme proteins that accelerate the reaction rates by a factor of 10^{10}-10^{23}. But the question of how enzymes work remains unanswered to this day.

This is where water enters the big picture [2]. It is well known that enzymes and other macromolecules, DNA and RNA, need a minimum amount of water in order to work at all, and much more to work efficiently. That is why cells are loaded with water, some 70 percent by weight. In terms of number of molecules, water far outnumbers all other chemical species - ions, small organic molecules and macromolecules – added together.

Water is needed for macromolecules to become flexible, in order to accomplish their otherwise impossible tasks of making sluggish chemical reactions happen spontaneously and effortlessly [1]. Recent computational and experimental studies have clearly shown that the flexibility of the proteins induced by water is important in reducing the free energy barrier between reactants and products [2]. The flexing of proteins also increases the probability of quantum tunnelling between reactants and products by a transient compression of the energy barrier. The same studies indicate that the dynamics of proteins are closely tied to that of water. Some would go as far as to say it is mainly the mobility of water that determines the magnitude of protein fluctuations, not only at the protein surface, but also in the protein core. But then the collective, conformational movements of proteins would feedback to influence the hydration water coherently [3].

The protein hydration shell, as generally regarded, is a single layer covering the surface of the protein, about 0.2g water per g protein, which enables it to function. The water is non-freezing, but undergoes a glass transition (a solid with no crystalline order) at about 170K, and is often compared to supercooled confined water [4].

At about 225 K, the protein undergoes a dynamic transition that some people believe, is a transition from a fluid high density liquid to a less fluid, low density liquid, or supercooled water.

Most of all, protein and hydration shell behave as one dynamically coherent unit from femto seconds to nanoseconds and beyond.

These results have been obtained with a combination of the most sophisticated techniques including x-ray absorption and emission, neutron scattering, dielectric spectroscopy, ultrafast infrared spectroscopy, combined with molecular dynamic simulations [2].

EXTENSIVE FERROELECTRIC HYDRATION SHELL

Over the past several years, Havenith and colleagues [5,6] used a new 'table-top' Terahertz absorption technology to provide evidence that the protein hydration shell is much more extensive than a single layer of water molecules.

They found that dissolving proteins in an excess of water led to a steep increase in absorption of Terahertz radiation, with the maximum absorption between 0.5 and 1 mM, when the protein molecules occupy an estimated 1 percent of the volume. Thereafter, the absorption dropped sharply and remained almost flat as protein concentration increased further. With the help of molecular simulations, they worked out that the protein's hydration shell extends 3-4 layers out from the protein surface, and when the average distance between proteins shrinks below that level as protein concentration increases, the Terahertz absorption also drops.

leBard and Matyushov [3] commented that to explain those observations requires a very large "effective dipole moment of the protein and its hydration shell, much exceeding the dipole moment of the protein itself." They confirmed, by numerical simulations, that the protein hydrations waters are polarized into a ferroelectric shell some 3-5 water molecules thick, with very large dipole moment and large amplitude fluctuations, much bigger than those in bulk water, in contradiction to the usual linear response theory. This was also demonstrated in real measurements.

There is even evidence of quantum effects and quantum coherence in the dynamics of the hydration shell and protein. One sign of quantum effect is the difference in behaviour observed when hydrogen (H) is substituted with its heavy isotope deuterium (D), as already clearly indicated for water itself [3,7].

Pagnotti and colleagues [7] used dielectric spectroscopy to compare the dielectric relaxation of lysozyme in ordinary water (H_2O) and in heavy water (D_2O) in the frequency range of 10^{-2} to 10^{7} Hz, and over the temperature range 210 K to 330 K.

If the effect is classical, there should be no difference between the sample in H_2O or D_2O. However, large differences were found. In particular, the dielectric relaxation times of H_2O were comple-

tely different from those measured with D_2O over the entire temperature range.

These observations were in close agreement with results of a sophisticated theoretical study using quantum mechanical methods from first principles.

In addition, the researchers reanalysed data from a deep inelastic neutron scattering experiment, which measured momentum distribution of protons n(p) and mean kinetic energy for a lysozyme sample prepared the same way as for the dielectric spectroscopy experiment. Measurements were made both above and below the protein's dynamic transition temperature (see above) at 290 K and 180 K respectively. They computed the minimum fraction of protons showing quantum behaviour that would produce the observed difference in the distribution of momentum between the two temperatures. This fraction of protons was 0.29, precisely the fraction present in the sample that belongs to the hydration water.

WATER DYNAMICS DOMINATES DNA

The fascinating story of DNA and water is just beginning [2]. Hydrating shells of DNA share many of the properties of hydration shells of proteins. In addition, water turns DNA into a electrical conductor and gives it magnetic properties; and many are looking into exploiting synthetic DNA in new molecular electronic devices.

Hydration water of DNA appears to have quite unusual dynamics as measured by time-resolved Stokes-shift (TRSS) experiments and confirmed by molecular dynamics simulation [8]. Hydration water governs the molecular dynamics of DNA with coherent vibrations from femtoseconds to nanoseconds that decay in a power law fashion, suggesting a high degree of correlation over all time scales.

The ability of DNA to conduct electricity has remained controversial for years. But Yamahata et al [9] have convincingly demonstrated that the ability of DNA to transport electrical charge depends on water. DNA bundles suspended in air between nanotweezers increased exponentially in conductivity up to 10^6 fold as the relative humidity of the air increased. The increase in conductivity was attributed to the rise in concentration of charge carriers as DNA hydration went up with relative humidity; the charge carriers being H^+ and OH^-, suggesting that proton (positive electricity) jump conduction could be involved as well as electron conduction.

An important finding is that the pathway for charge transfer (conduction) is through the stacked bases in the core of the double helix where the ω molecular orbitals overlap. Berashevich and Chakraborty [10] used quantum chemical calculations to demonstrate that interaction of the bases with water (see Fig. 1) breaks some of the π bonds in the ring structure of the bases, giving rise to unbound π electrons. These unbound electrons, together with the change in DNA conformation from A (dehydrated) form to B (hydrated) form, could account for the exponential increase in conductivity as relative humidity and hydration of the DNA molecule increases.

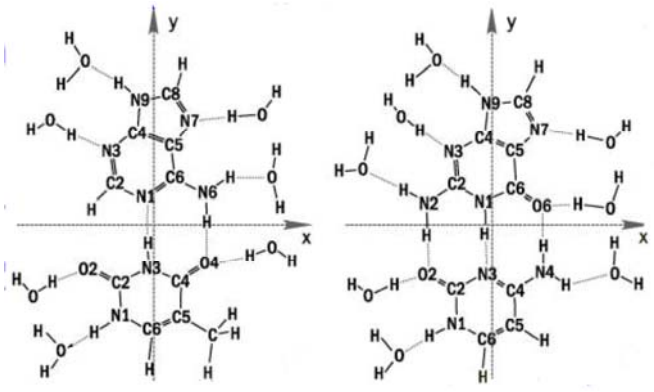

Figure 1. Water binding to DNA bases [10]

At low temperatures, the efficiency of charge transfer is determined by the spin interaction of two unbound electrons located on neighbouring bases of the same strand. Exchange is allowed only when the electron spins are antiparallel (in opposite directions). Hence the conductance of DNA can be controlled by a magnetic field. This gives the potential for developing nanoscale 'spintronic' devices based on the DNA molecule, where the efficiency of spin interaction will be determined by the DNA base sequence.

Regardless of the practical application, these findings have profound implications for the biological functions of DNA, apart from serving as a linear code for the sequence of amino acids in proteins. We are only touching the tip of a very large iceberg.

THE RAINBOW ENSEMBLE

We now venture inside the cell and see how everything, all the ions, macromolecules and other molecules, which I shall call the rainbow ensemble, can dance together to produce life [11].

Decades of research led to a general agreement concerning the interaction of ions with water in terms of kosmotropes and chaotropes [12]. Kosmotropes bind strongly to water, so they end up with more bound water in their solvation shell, chaotropes bind weakly to water, and have very few if any water molecules bound.

But ions in water also affect proteins in solution. Frank Hofmeister, a Czech scientist in the late 19th century, found that some salts helped egg white proteins to dissolve in water, while others caused the proteins to precipitate out, and there were those that had effects in between. He ranked the ions according to their ability to "salt-out" and "salt-in", which resulted in the Hofmeister series. The Hofmeister series is correlated with other properties, but until quite recently, there has never been a satisfactory explanation.

Kim Collins may have found the answer, and it is related to the ions' affinity for water [13,14].

When pairs of oppositely charged ions have similar affinities for water, something special happens: they come out of their solvation shells, join up and neutralize each other. That's because they can just as easily form intimate partners with each other as with water molecules; exchanging water molecules for the counter-ion does not cost anything in energetic terms. This 'Law of Matching Water Affinities' appears to explain why certain salts are less soluble than others, and why some salts precipitate proteins out of solution while others help them dissolve. The answer is that only neutral molecules precipitate (or crystallize) out of solution; neutral molecules have much lower solubility.

More specifically, a radius of 1.06 Å separates small monovalent cations from large ones, and a radius of 1.78 Å separates small monovalent anions from large ones. Small monovalent ions are strongly hydrated, while large monovalent ions are weakly hydrated. For example, LiF contains small monovalent ions that readily come out of their hydration shells to pair up as 'contact ion pairs', it has a solubility of only 0.1 M. In contrast, CsF has a large cation and a small anion, and do not pair up in solution; it has a solubility of 24.2 M.

The same principle applies to ions carrying two or more charges. For polyatomic ions, water affinity is determined by the surface charge density of the specific atom that water interacts with. This simple hypothesis seems to account well for the salting in and salting out of proteins.

Most of all, Collin's theory shows that the intracellular concentrations of ions – which are opposite of those in extracellular fluids – are optimised for keeping proteins and other macromolecules in solution. Intracellular fluid has high concentrations of potassium and magnesium cations and phosphate and sulphate anions, and very low concentrations of sodium and chloride; the converse is true of extracellular fluid: low in potassium, magnesium, phosphate and sulphate, and high in sodium and chloride. While there appears to be not much difference between extracellular and intracellular calcium, most of the intracellular calcium is bound, with only 10^{-7}M free Ca^{2+} most of the time, except for very transient, local increases associated with signal transduction.

Apart from the inorganic ions, there are some 65 mM of proteins present in the cytoplasm, which are rich in carboxylate anions in their side chains. As Collins pointed out, the intracellular ions are optimised for *mis*match in water affinities, so as to maintain high solubility of the proteins and other constituents of the cytoplasm at all times.

Increasingly, protein-folding disorders are being identified, including Alzheimer's disease, Parkinson's disease, transmissible spongiform encephalopathies (mad cow disease), Huntington's disease, and type II diabetes, which have been linked to ligand binding and hydration [15]. In all likelihood, these diseases represent different failures in keeping almost all the molecular participants in cellular biochemistry dancing with water at any one time, so some of them end up salting out at inappropriate places.

In view of the high affinity of sodium ions for carboxylate, intracellular concentration of sodium is kept very low; and it is generally believed, by a Na^+/K^+ ATPase that pumps sodium out of the cell in exchange for potassium.

Is that really so?

THE BIG MYSTERY OF LIFE REMAINS

How did the cell manage to have just the right combination of ions inside, which is completely the opposite of what's on the outside? How could the cell have evolved before it acquired all the complicated pumps and channels to keep all the right ions inside and the wrong ones outside?

Physiologist Gilbert Ling has stubbornly rejected the idea that it was all due to membrane pumps. Instead, he has maintained for more than half a century that the cytoplasm naturally excludes sodium and binds potassium [16–19]. His association-induction hypothesis proposes that the major components of living protoplasm – water, proteins, and K^+ - exist in a closely associated, high-energy state at 'rest'. Purified proteins, he said, are not at all what proteins are like in the cell. Instead, within the cell, most if not all proteins are extended so that the peptide bonds along their polypeptide chains are free to interact with multiple layers of polarized water molecules and their carboxylic side chains similarly preferentially bind K^+ over Na^+. The other rea-

son is the ubiquitous presence of ATP in the living cell.

Living protoplasm is full of ATP, which is bound to proteins at certain 'cardinal sites', according to Ling. These ATP-bound sites then induce changes in the electron density, ultimately of the entire polypeptide chain, including the side chains.

In the absence of ATP, proteins do tend to adopt secondary structures - alpha helix, or a beta pleated sheet - due to hydrogen bonding between peptide bonds in the same chain, which gives them a folded up conformation where they don't interact maximally with water. However, when ATP is bound to the cardinal site, it tends to withdraw electrons away from the protein chain, thereby inducing the hydrogen bonds to open up, unfolding the chain and enabling it to interact with water.

For me, among the most persuasive evidence was the experiments Ling and his co-workers performed, showing that cells with cell membranes made permeable with detergents, or completely cut off at one end, nevertheless maintained their distinctive ionic compositions over long periods of time.

WHAT'S THE CELL REALLY LIKE?

It is necessary to look at the cell again, not as a membrane bound entity containing various organelles suspended in an otherwise featureless cytoplasm, still widely supposed to consist of proteins in aqueous solution [19]. That view was already strenuously refuted by Joseph Needham who cited extensive evidence in his book, *Order and Life* first published in 1936 in support of meticulous molecular organisation in living *protoplasm* [20]. Living protoplasm differs strikingly in many respects from the same proteins dissolved in water, including the ultraviolet absorption spectra. This book also suggested that living cells are polyphasic liquid crystals, anticipating the discovery in my laboratory by 56 years.

Welch and Clegg [21], lead champions of the view that the cytoplasm is organised; recently published an important review on the history of protoplasm. They recall how Keith Porter used high voltage electron microscopy to show up the 'microtrabecular lattice' (MTL) of the cytoplasm, which provides scaffolds for enzyme and other proteins, as well as numerous micro-environments or nanospaces for specific enzyme reactions to take place independently of one another.

Much later, the MTL was rediscovered as the ubiquitous cytoskeleton composed of filaments made of actin and other proteins, a dynamic structure that's constantly breaking down and reforming as the cell changes shape, and transports materials to all parts of the cell. There is at least one journal entirely devoted to the topic of intracellular transport [22], and it is full of photographs of cells with cytoskeleton lit up in a fine meshwork of neon fibres stained with fluorescent antibodies for the cytoskeletal proteins.

There is good evidence that water exists as a mixture of two states at ambient conditions, high density water and low density water. According to Philippa Wiggins, high density water and low density water has preferential affinities for kosmotropes and chaotropes respectively [23,24]. Wiggins has further suggested that the spontaneous inter-conversion of these two forms of water in microdomains inside cells or enzyme active sites may be what gives life its seemingly boundless 'free energy'.

Martin Chaplin has proposed a similar scheme incorporating Ling's induction-association hypothesis [25]. A relatively static (resting, or poised for action) regime in low density water associated with ATP and K^+ carboxylate binding and actin polymerization alternating with a more mobile high density water regime of actin depolymerisation and less K^+ carboxylate binding.

These hypotheses are amenable to experimental testing. For example, no one has yet tested whether proteins do behave differently in solutions of K^+ and Mg-ATP. Or whether proteins enclosed in phospholipid micelles might behave differently and show selectivity for K^+ over Na^+ due to a change in the state of water.

Most of all, we must always bear in mind the hauntingly beautiful intimation of what the living cell is really like when Ludwig Edelmann at Saarland University in Hamburg, Germany, took extraordinary care in preserving its fully hydrated living state (Fig.2).

CONCLUSION

Recent advances in analytical techniques have brought us tantalizingly close to solving the age-old mystery of life, confirming in fine detail how cells and organisms depend on quantum coherent molecular energy machines, as predicted by physiologist Colin McClare some 40 years ago (see [1]). They give new insights into how such quantum coherent molecular energy machines, organised in microdomains, are orchestrated by the liquid crystalline water of hydration switching effortlessly between low and high density states to provide the 'engines of life'. However, the precise nature of living protoplasm remains just beyond our grasp.

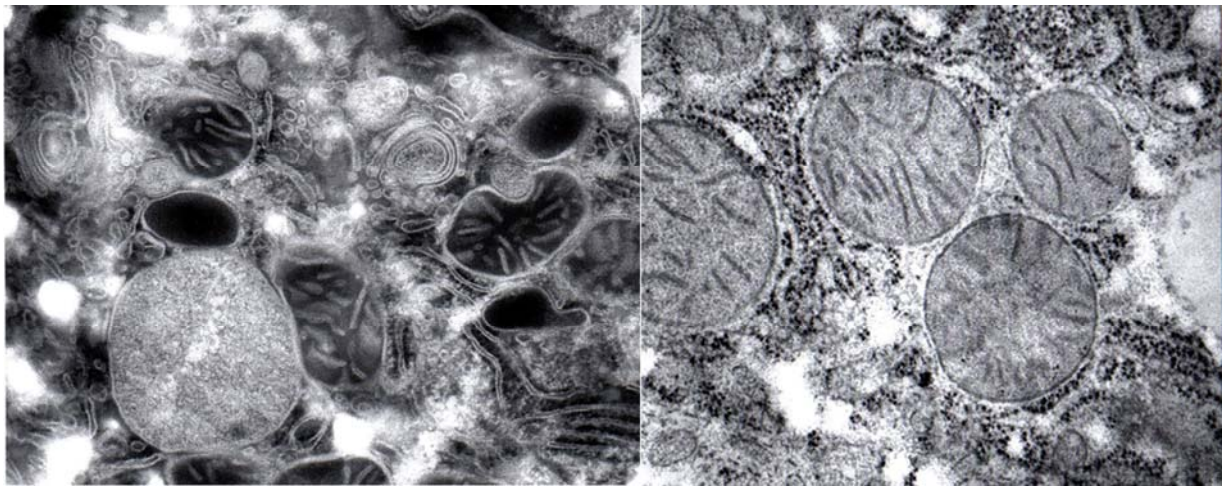

Figure 2. The fully hydrated cell (left)as captured by Edelman, and the dehydrated cell (right) in conventional electron microscopy

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ТЕЧНА КРИСТАЛНА ВОДА, КВАНТНЕ МОЛЕКУЛАРНЕ МАШИНЕ И ЖИВИ ОРГАНИЗМИ

Апстракт: Прошло је 18 година од открића у мојој лабораторији, које показује да су живи организми динамичне ликвидне кристалне фазе у којима су све молекуле у ћелијама и ткиву организма поредане и заједно се кохерентно крећу, а нарочито молекуле воде које су својствене течној кристалној матрици и суштински су важне за функцију макромолекула. У ствари, организам је заснован на макроскопској квантној кохерентности појединачних молекуларних енергетских машина.

Недавна испитивања у којима је коришћено неколико софистицираних спектроскопских техника заиста су показала да течна кристална вода формира динамички кохерентне јединице са протеинима и нуклеинским киселинама, и омогућава макромолекулама да заједнички кохерентно функционишу.

Иста истраживања такође показују да јони играју кључну улогу у ћелијском метаболизму и слању сигнала путем интеракције са хидратном водом. Упркос овом великом напретку, крајња мистерија живота остаје задивљујуће изван нашег схватања.

Кључне ријечи: течни кристални организам, квантна кохерентност, молекуларне енергетске машине, хидратна вода, два стања воде, козмотроп и каотроп, закон афинитета измјењивих молекула воде, микродомени.