Physiol. Chem. Phys. & Med. NMR (2012) *42*: 1–64

What Is Life Answered
in Terms of Properties and Activities of Auto-cooperative Assemblies of Molecules, Atoms, Ions and Electrons Called Nano-protoplasm

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KEY WORDS: AI cascade mechanism; Alan Chalmers; anti-science; association-induction hypothesis; atoms; ATP; auto-cooperative adsorption; c-value; c-value anlogue; Ca^{++}; cardinal adsorbent; cardinal site; cell; chlorine ion; cooperative phenomena; death; drugs; drug design; electrons; H^+; hemoglobin; hormones; hydrogen ion; K^+; Kinetic Theory of Gases; life; life activity; living; living cell; macroscopic protoplasm; microscopic protoplasm; molecules; Na^+; nano-protoplasm; ouabain; polarized-oriented-multilayer theory of cell and model water; PM theory; POM theory; potassium ion; proteins; protein folding; protoplasm; Queen Victoria's transistor radio; Salt-Linkage Hypothesis; Schrödinger; Science; science philosophers; Scientific Method; sodium ion; sodium pump; two-state model; What is this thing called science?; Yang-Ling Cooperative Adsorption Isotherm.

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Abstract of background and content

IN MID-17TH CENTURY English chemist, Robert Boyle regretted: "It is highly dishonorable for a reasonable soul to live in so Divinely built a mansion as the Body she resides in, altogether unacquainted with the exquisite structure of it." After the Lisbon earthquake in 1755 where many innocent men, women and children were killed for no faults of their own, French writer Voltaire lamented in his poem: "Man is a stranger to his own research…thinking atoms …have measured distant stars,…Ourselves we never see or come to know." In 1738 and thus seventeen years before Voltaire invoked thinking atoms, Swiss-Dutch mathematician, Daniel Bernoulli published his Kinetic Theory of Gases. In this theory gases are collections of rapidly and randomly moving corpuscles or molecules and their ceaseless bombardments on the wall of the container give rise to the pressure in all directions—a phenomenon unexplained until then. Notwithstanding, he and three other top-notch advocates of the Kinetic Theory of Gases were all rejected. Indeed, physicists as a whole did not believe that atoms (and molecules) are real until the beginning of the 20th century—some 170 years after Bernoulli's publication. The present paper mentions how the last rejected creator of the Kinetic Theory of Gas, the ill-fated Austrian mathematician-physicist, Ludwig Boltzmann also invented the modern branch of physics called Statistical Mechanics before taking his own life. And it was this invention and the new knowledge on protein structure uncovered by the German chemist, Emil Fischer that had provided the twin pillars, on which "A Physical Theory of the Living State" called "the Association-Induction Hypothesis" was launched in 1962. Another sixty years of continued theoretical and experimental studies later, I am ready to present here the most up-to-date and mature version of the only known definition of life. Thus, at long last Mankind has come to know what we are in our divinely built Mansion.

1 Introduction

In the title of a booklet he wrote, one of history's greatest physicists, Erwin Schrödinger (1887–1961) asked the question "*What is Life?*" (Schrödinger 1944.) That booklet has been reprinted by the Cambridge University Press at least 18 times and must have sold thousands upon thousands of copies. Notwithstanding, Schrödinger only asked but did not answer the question he posed. Francis Crick of the DNA fame was more straightforward. In his 1981 book, "*Life Itself, its Origin and Nature*," he wrote "that it is not easy to give a compact definition of either *life* or *living*" (Crick 1981 p. 49.)

To make certain that I did not overlook any existing cogent answer(s) to the question what is life, I quote below the definitions of the word, life, from six dictionaries, ranging from the elementary to the advanced, from the traditional to Wikipedia, which is being steadily updated.

Thorndike and Barnhart: High School Dictionary

(Life is the) Quality that people, animals and plants have and that rocks, dirt and metals lack.

Webster's New Collegiate Dictionary, 1977

1. (Life is) The quality that distinguishes a vital and functional being from a dead body.
2. (Life is) A principle or force that is considered to underlie the distinctive quality of animate beings.
3. (Life is) an organismic state characterized by capacity for metabolism, growth, reaction to stimuli, and reproduction.

Webster's New Twentieth Century Dictionary (unabridged), Second Edition, *1968*

1. (Life is) That property of plants and animals which makes it possible for them to take in food, get energy from it, grow, adapt themselves to their surroundings, and reproduce their kind: it is the quality that distinguishes a living animal or plant from inorganic matter or a dead organism.
2. (Life is) The state of possessing this property as we tried to bring the drowned child back to life.

The Oxford Universal Dictionary on Historical Principles. 3rd and Revised Ed. 1955

1. (Life is) Primarily, the condition, quality or fact for being a living person or animal.
2. More widely, (life is) the property which distinguishes a living animal or plant or a living portion of organic tissue, from dead or non-living matter; the assemblage of the functional activities by which the presence of this property is manifested.

Wikipedia, the Free Encyclopedia, July 15, 2012

Life is a characteristic that distinguishes objects that have signaling and self-sustaining processes from those that do not.

The American Heritage Dictionary of the English Language, 4th edition

1. (Life is) The property or quality that distinguishes living organisms from dead organisms and inanimate matter manifested in function such as metabolism, growth, reproduction and response to stimuli or adaptation to the environment originating from within the organism.
2. (Life is) The characteristic state or condition of a living organism.

The definitions given by the six dictionaries for the word, life, are most frequently expressed as *a property, a quality, or a state* and less frequently as a principle, a force, a characteristic, a condition, a fact or activities. However, none tells us even briefly what that property, quality, state etc. is in terms of the laws of physics and chemistry that govern the dead world.

Given the pervasive ignorance on what life is worldwide, how can a reader reconcile it with the title of this article *What Is Life Answered* and the content of its abstract to the same effect? When the reader reaches the end of this communication, he or she will know the full story of the cause of this apparent contradiction. But before that I offer a pointer.

Beginning in mid-20th century, the continued effort of a tiny minority of our species has succeeded in understanding in modern physico-chemical terms what distin-

guishes the vast amount of dead matter that makes up virtually the entire Universe—from the least amount of matter that makes up a bacterium, a rose, a nightingale as well as you and me: ***life****.*

Only the new truth found by this tiny minority is still unknown to the vast majority of our kind, nor taught in high school and college courses across the whole world with one lone exception—in Gifu University of Japan under the direction of Prof. Hirohisa Tamagawa. The main cause for this delayed response is not hard to imagine if one looks for it in the past history of science. For example, it took one hundred and seventy (170) years for the physicists to accept the (revolutionary) Kinetic Theory of Gases and the reality of atoms and molecules. Our delay has not reached that vintage yet. Nonetheless, a part of this communication is devoted to making this "dark age" as brief as possible. And to achieve that goal, I shall start with a question.

1.1 Principle of sequential invention

To assess the long-term outcome of a prolonged delay of the adoption of one of the most relevant of all basic knowledge to the long-term welfare of Mankind, I turn to the Website that I introduced earlier (Ling 1998.) It bears the title, "*Science Cannot Cure Cancer and AIDS without Your Help.*"

In this Website, I started with a question embedded in a parable: Could Queen Victoria of England at the height of her power and her entourage of brilliant minds repair a transistor radio, which I sent her by magic and it broke? My answer was an emphatic no—not even if she is willing to enlist all the capable scientists in the world and to spend every shilling in the Treasury of Great Britain. There was no way for anyone to fix the faulty operation of something at a time when no one knew that something existed (Ling 1974; Ling 1992 p. xxii; Ling 1998.)

Yet, once we understand how electrons work in a radio, it would cost next to nothing to have a broken one restored to normal function. Thus the invention of something epochal but complex can only follow the understanding of the underlying basic knowledge. For convenience of reference, I named the underlying principle, the ***principle of sequential inventions*** (Ling 1998 p. 3.)

Thus fifty years after Michael Faraday discovered magneto-electric induction (1831) the first electric power plant came into existence (1880.) Thirty years after Maxwell introduced his unified theory of electromagnetic waves, (1867–1873,) Marconi obtained a British patent for the future radio industry (1900.) Notice also that the discovery of one relevant basic truth spawns not just one useful product but an ever-expanding tree of other basic knowledge and their respective useful products. The universal rule is that the more relevant and the more basic a new discovery, the wider is its beneficial impacts on the current and future welfare of all Mankind. From the vantagepoint of humanity, no basic knowledge could be more relevant and more basic than what life is in terms of the laws that govern our Universe.

By the same token, we cannot cure deadly cancers for a similar reason that Queen Victoria could not fix her broken radio. Only in this case, it was not the absence of relevant basic knowledge but the reliance on an entrenched, *erroneous* basic theory of life, called the membrane theory and/or membrane pump theory, that have been road-blocking what could be unprecedented progress in biomedical research and education.

In both the membrane theory version and the membrane pump theory versions, the basic units of life called cells are membrane-enclosed tiny sacs of watery solutions comprising

ordinary liquid water, fully dissociated ions and so-called "native proteins" (see Ling 2006 for reasons why the so-called "native proteins" are not native.) And both versions have been thoroughly disproved. Thus the **membrane (pump) theory** has been disproved by (at least) three sets of independent evidence published between 1962 and 1980 (Ling 1998a): (1) 1500% to 3000% energy insufficiency to operate just one pump at the cell surface, the sodium pump (Ling 1962; Ling 1997), (2) squid axon membrane sacs with its cytoplasm replaced by a watery solution of the right composition do not exclude Na^+ or accumulate K^+ (Ling and Negendank 1980), (3) muscle cytoplasm without functional cell membrane and postulated pumps excludes Na^+ and accumulates K^+ (Ling 1978; Ling 1997.) With equal thoroughness, has the alternative **membrane theory** been disproved in theory and via a multitude of experimental testing (Ling 2011.)

If the disproof of the still widely taught membrane or membrane (pump) theory were all I could tell you, it would give little comfort to anyone. *After all, it might take decades if not centuries to invent and then prove valid an alternative new theory to replace the wrong one.* Ironically, the virtually unknown achievement alluded to above holds the key to a happier and more secure future for all Mankind. But as also mentioned briefly above, to reach that long term goal we must first find ways by which the new truthful knowledge (and other revolutionary truths yet to come in the future) can be taught widely and soon. To reach that objective, we need to unearth whatever unconcealed as well as concealed "Trojan horses" that threaten the spreading of newfound knowledge.

1.2 The buried knowledge

A man unexpectedly inherited a big bag of silver. Worried that others would steal it, he buried the bag underground. However, even that did not stop him from worrying. Maybe the site is too close to a busy street and that means danger. For what he thought would add more safety, he put up a big sign over the burial site, announcing that "***there is no buried treasure of 300 taels of pure silver at this location***." Not long after that, a passerby saw the sign. Distrusting the reliability of the one that put up such a sign, the passerby got a shovel and started digging. Soon he found the bag and walked away with the 300 taels of silver.

This is, of course, an old Chinese story. Believe it or not, it has a modern counterpart. Only it was not another Chinese character that did the repeat but the British corporation that publishes the ***Economist*** magazine (with its alleged global circulation of 1473937.) Thus its Technology Quarterly Section of its Dec. 5, 2003 issue contained an article entitled "MRI's Inside Story". In this article, the magazine announced to its worldwide readership: "*Following an obscure theory by Gilbert Ling, a physiologist…Most scientists consider Dr. Ling's ideas as wacky at best*." (*Wacky* is slang for irrational, crazy according to the Webster Dictionary.) A passerby saw this announcement. He too suspected the magazine had something to hide and began digging. What he found was a collection of scientific publications on or about life including:

> ***"A Physical Theory of the Living State: the Association-Induction Hypothesis***", a 680-page long monograph published in 1962 by the Blaisdell Publ. Co., a branch of Random House Publishing Co. of New York;
>
> ***"In Search of the Physical Basis of Life***", a 791-page long monograph published in 1984 by the Plenum Press of New York and London;

> ***"A Revolution in the Physiology of the Living Cell"***, a 378-page long monograph published in 1992 by the Krieger Publishing Co. of Melbourne, Florida;
>
> ***"Life at the Cell and Below-Cell Level: The Hidden History of a Fundamental Revolution in Biology***", a 373-page long monograph published in 2001 by the Pacific Press of New York.

Not to mention more than 200 scientific reviews and full-length articles on subjects related to life and living—published one after another in established scientific journals mostly in the US and UK over a long span of time. Thus a new unifying theory of living phenomena, called the *association-induction hypothesis*, was introduced half a century ago. And it has been extensively tested and confirmed worldwide—without a single major setback. *And imbedded in the four books and other documents published over half of a century, is an evolving theory of life in physico-chemical terms*.

What is more, 15 years after its introduction, the association-induction hypothesis had led Raymond Damadian to invent the medical technology known as *magnetic resonance imaging* or MRI. And here is how Dr. Damadian described that moment of history in a letter to me dated November 9, 1977 (Ling 1992 p. xxv; Ling 1984 p.vii.)

> "On the morning of July 3, at 4:45 A.M….we achieved with great jubilation the world's first MRI image of the live human body. The achievement originated in the modern concepts of salt water biophysics, on which you are the grand pioneer with your classic treatise, the association-induction hypothesis." (Ling 2001 p. 83.)

Thus, Damadian's invention has confirmed once more the *principle of sequential inventions* I introduced and mentioned above. But that was not all. Damadian's comments on the association-induction hypothesis also shine light on a big medium's total disregard of the good name of people who have done them no harm. Indeed, the potential damage it has done by its reckless abusiveness is matched only by its inability to tell truth from lies.

Not long after I heard about the attack, I wrote to the top brass of the magazine. I asked them if they had actually interviewed the majority of the world's scientists and got everyone of them to divulge their assessment on my scientific ideas as irrational and crazy or *wacky at best*? And if so, where is their published evidence—when in fact I could not find a trace of such evidence in the literature? Though a separate set of my letter and supportive documents was sent respectively to the President, the Board Director, the Editor-in-Chief of the magazine, not one answered or told what they had actually done and not done, and apologized publicly.

A detailed account of this bizarre episode including a reasoned guess at its immediate motivation for mounting the attack is published in the same issue of the journal publishing this one (Ling 2012.)

Done with one example of how one wide-circulating magazine interferes with the normal spreading of newfound scientific knowledge by lying to the public about my scientific reputation, I now approach a broader and sustained attack on science and even the existence of truth.

1.3 The Scientific Method and what it can and cannot do

For a long time, most practicing scientists shared the belief that the set of step-by-step procedures to find truth and called the Scientific Method was invented in the West. In fact, this belief is totally wrong. The Scientific Method was invented by an Arab, Ibn al-Haytham or (its Latinized version) Alhacen, who lived in the Islamic Golden Age between

965 and 1040 (Alhacen 2013.) The West did not adopt the Scientific Method until the post-renaissance or early modern period. And then falsely attributed its invention to Galileo Galilee, René Descartes, Robert Boyle and others.

The more organized truth-seeking that followed the adoption of Scientific Method has been known as *modern science* (or simply science)—to be distinguished from the earlier disorganized intellectual effort called *natural philosophy*. A unique gift that the Scientific Method has given to modern science is a way of experimentally *falsifying* a hypothesis. Nothing like it had existed before.

By making it possible to determine if one's own hypothesis or that from others has validity, the Scientific Method had also transformed the search for truth from one of isolated individual activities to the cooperative activities of an open-ended group of individuals or groups of individuals worldwide.

However, to carry out communication far and wide was no easy task at that time. To answer this and related needs, the immensely wealthy Islamic caliphs had built the famous edifice called the *House of Wisdom* in Bagdad, a city itself then newly-erected on the bank of the Tigris River. Nominally referred to as a library, the House of Wisdom was much, much more. To begin with, the caliphs gathered needed "tools" from remote sources. Thus they learned how to make (cheap and foldable) paper as well as printing from China and "Hindu-Arabic numerals" from Hindu. They also bought or otherwise obtained recorded knowledge from diverse surviving or rapidly-vanishing old culture and had them all translated into a single language, Arabic. And then printed them on paper to be bound into books or pamphlets—made available at easily accessible at the library of the House of Wisdom and elsewhere in the Islamic Empire. Then just as quickly the Islamic Golden Age ended. Only then, modern science became the foster child of European West. Years passed.

1.3.1 Science philosophers and anti-science

Far down the time line, a small group of individuals who lived and died in the second half of the 20th century appeared on the scene and called themselves Science Philosophers. Often referred as a group, they are Karl Popper (1902–1994), Thomas Kuhn (1922–1996), Paul Feyerabend (1924–1994) and Imre Lakatos (1922–1974.) In my guess, their immense visibility and influence could be traced to the fact that each top-ranking university across the world usually had a Department of Philosophy. However, a main subject of philosophy had been the philosophy of Nature—a subject matter that had been taken over by Modern Science and taught in newly installed science departments. In consequence, the old philosophy departments were left with less and less subject matter for teaching and research. So when some individuals came along and proclaimed that they were masters of both science and philosophy, these individual were snatched up fast. In support of this explanation, I may mention that when Paul Feyerabend turned 46, he was offered professorships in no less than ten top universities of the world, including the Berlin University in Germany, Yale University of the US and Oxford University in Great Britain (Preston *et al* 2000.) Unfortunately, the overall legacy the quartet of science philosophers left behind is not what one would hope it to be—with the possible exception of the Hungarian mathematician, Imre Lakatos (Lakatos 2013.)

In what follows I shall comment on some specific ideas and pronouncements of Popper and Kuhn. As far as Feyerabend is concerned, when he proclaimed that science

cannot prove or disprove a hypothesis and that "everything goes," (Theocharis and Psimopoulos 1987 p. 596), my answer is simply that he was totally wrong as will be made clear below.

1.3.1.1 THOMAS KUHN

In 1962 Kuhn published "The Structure of Scientific Revolution" and became famous (Kuhn 1962, 1970.) Among many of his admirers was myself. (Ling 1992, p. 319.) The reason I thought so highly of his work at that time was that he stood apart from the other influential scientists who insisted that progress of science is linear. That is, new ideas all came directly from past science, thereby justifying the deployment of the *peer review system, in which establishment scientists or peers decide who get public support and who don't* (Ling 1998b.) To insist that scientific progress is linear, these scientists openly ignored historical facts. That is, science can progress smoothly but from time to time, it may also undergo drastic revolutionary changes (see Ling 1998c.) Unfortunately, Kuhn did not just highlight the existence of these revolutionary changes, he also separated scientists into two classes: revolutionary scientists are like eagles in the sky and normal scientists are like barnyard fowls. In consequence—in my view, he got so much flak that he overresponded by turning against revolutionary science—claiming successive revolutionary changes do not bring its investigator(s) closer to truth but only from one fad to another fad. It is this changing from talking about truth to talking about nonsense that had made his overall contribution to science and society anti-climatic and harmful (Theocharis and Psimopoulos 1987; Theocharis 1987. See also Horgan 1996.)

1.3.1.2 SIR KARL POPPER

Sir Karl Popper (1902–1994) was born in Austria, he later taught at the University of London and was knighted in England. It is widely known that according to him, science can only disprove a hypothesis but it cannot prove a hypothesis (Popper 2013.) On that I totally disagree. (See Section 1.3.2 below for reasons.)

1.3.1.3 ALAN CHALMERS

Unlike the quartet of science philosophers described above, Alan Chalmers is still alive and vigorously active. Born in Bristol, England in 1939, he got his Ph.D. from the University of London. While there he apparently came under the influence of Sir Karl Popper. In 1971, Chalmers went to Australia and began working at the University of Sydney. As of this time in 2013, he is working on the 4th edition of his enormously popular best-seller textbook, "*What Is This Thing Called Science*?" So far, this book has already been translated into fifteen languages.

"*What Is This Thing Called Science*?" first appeared in 1976. It was then sold as a textbook for an introductory university course on the philosophy of science. The heading of the last section of its second 1982 edition of the book reads "Why Bother?"

Dr. Chalmers also proclaimed that the most important function of his book is to combat the *Idealogy of Science,* which he saw as the insistence of *perpetrating the dubious concept of science and the equally dubious concept of truth.*

While I am not sure this was his original intention, he did write early on in the Introduction of the above-mentioned book the following passage: "We start off confused and end up confused at a higher level."

In their rebuttal of Chalmers's claims in the Nature magazine's Commentary, Theocharis and Psimopoulos together (1987) or Theocharis alone (1987) pointed out that it is only on true knowledge that the socially beneficial and economically profitable medical and technological applications can be firmly grounded. And true knowledge is often discovered by the judicious application of the Scientific Method. Indeed, this is a different way of expressing what is expressed in the *principle of sequential inventions* I presented earlier.

Notwithstanding, the anti-science movement has become so popular not only with the public but worse, it has become just as popular among the professional philosophers and scientists—not to mention teachers who buy and teach what is in such a highly popular textbook. Theocharis and Psimopoulos ended their commentary with the plea that scientists and philosophers stop running down their own profession and start fighting for their causes earnestly.

As a professional scientist all my life, I have always felt it an unexcelled privilege to have the opportunity to offer the foundation truths for more security and happiness of all members of our species in time to come. In my view, to deny that unexcelled privilege to our younger generations is bad beyond words. In direct opposition to Popper, I shall also demonstrate that a scientist can find old as well as new ways to prove a hypothesis with variations of the Scientific Method—as I try to do in the section immediately following.

Come to think about it, I suspect that different versions of what I am going to describe below could have been in practice all along. How else are you going to tell me that most serious-minded research scientists spend their time? Merely producing hypothesis that could only be disproved but never proved? If that were the case, before too long, the scientific laboratories worldwide would be filled with discarded scientific hypotheses and nothing else. That, of course, did not happen.

1.3.2 Proving a hypothesis via variations of the Scientific Method

To begin, I should remind the reader that the Scientific Method is limited in practice to deal with observations that lend themselves to experimental testing. You can do an experiment on rats but you cannot do an experiment on the Black Hole. Notwithstanding, scientific hypotheses that do not lend themselves to experimental testing can and have been proved with the aid of non-experimental methods. By variations of the Scientific Method, one can also prove observations that do lend themselves to experimental testing, as I shall also demonstrate below.

1.3.2.1 PROOF BY MATHEMATIC METHODS

That the square of the length of the longest side of a rectangular triangle equals the sum of the squares of the respective length of its two shorter sides is a scientific truth introduced independently by Pythagoras of ancient Greece and by an ancient Chinese scholar. And each had provided a distinctly different proof (Brownowski 1973.) The one often seen in Encyclopedia and easier to follow came from China (given in the treatise, Zhou Bei Suan Jing.) Thus both the ancient Greek and ancient Chinese have done what Popper (and Feyerabend) thought impossible: proving a hypothesis.

1.3.2.2 PROOF BY IMPROVING THE METHODOLOGY

The hypothesis is that the earth is round or better, spherical. Ferdinand Magellan unwittingly conducted an experiment proving that the earth is indeed round or spherical by sailing steadily westward until his surviving crew returned to the place where they started

from, Seville of Spain. Popper, however contended that this is a hypothesis that cannot be falsified (Theocharis and Psimopoulos 1987 p. 595, Col. 3.) I disagree. It would be falsified if Magellan and/or his crew sailed into empty space off the edge of the flat earth.

However, there is a valid and but different reason that Magellen and his crew might not have proved that the earth is spherical: for the returning crew could have proved the alternative hypothesis that the earth has the shape of a cone or a cylinder that is continual in less than three dimensions. But then all we need is to find a better experimental technique to conduct a different experiment. This time, it was a spacecraft rather than a ship sailing on the ocean waves.

Thus on December 11–12, 1990, the spacecraft Galileo took advantage of the solar eclipse ongoing on those days, and shot many pictures including a movie of the earth turning around and around revealing all sides of the sphere. We then have succeeded in proving that the Earth is spherical.

1.3.2.3 PROOF BY DISPROVING ALL OTHER ALTERNATIVE HYPOTHESIS

Suppose you were camping and found local mosquitoes were making your vacation intolerable. Think hard and you would probably reach the conclusion that there are only three ways to protect yourself: (1) install a mosquito-proof screened enclosure or net; (2) remove the pests by killing them or catch each pest that gets close and release it at a far-away location; (3) spray the near-by space with something that the mosquitoes do not like: a repellent. In fact the equivalent of each of these models has been proposed by cell physiologists to keep Na^+ level low inside the living cell. They are respectively:(1) the sieve theory; (2) the sodium pump theory and (3) the polarized-oriented multilayer (POM) of cell water (which partially excludes Na^+.)

Radioactive tracer studies have shown that Na^+ in fact traverses the cell membrane with ease, disproving model 1; energy inadequacy disproved model 2, leaving only the third choice still intact. In fact, the prior exclusion of the only existing alternative competing theories has proved the validity of the only known alternative, namely the POM theory of cell water as an integral part of the AI Hypothesis.

However, if someone claims to have found a fourth mechanism to get rid of mosquitoes or to partially exclude Na^+, it would most likely prove invalid because the POM theory has already many converging proofs—see Section 1.3.2.5 below. This is the beauty of the truth: there is only one.

1.3.2.4 PROOF ON A NON-ENDING LIST OF MODEL SYSTEMS

When the fragility and/or complexity of a living system forbids proving a hypothesis experimentally directly upon it, one can find or invent cogent models and test the hypothesis experimentally on these less fragile objects. Indeed, there is almost no limit on how far one can go in this general direction. For an example, see Section 5.2 below.

1.3.2.5 PROOF BY CONVERGING EVIDENCE

Confirmation of our hypothesis by our own laboratory or by other laboratories using similar or different techniques is an important step in proving a hypothesis on phenomena that are highly complicated like life. Two other variations would further enhance the validity: (i) retroactive confirmation from experimental studies carried out in the past by investigators who had no idea of the theory yet to evolve; (ii) experimental data that came as refutations of the theory but when carefully examined with or without new data turned out to be supportive evidence.

2 The great breaks that paved the way

As mentioned above, I take great pride in my role in the scientific accomplishment summarized in the title of this article. I also believe it an unexcelled privilege to do what I have been doing all my life.

For a start, I mention one specific happy event of my life: I came to the United States to study cell physiology right after the end of WWII. I could do this because I had won shortly before a nationwide competitive examination in China. It was a competition for the (one) biology slot among 22 so-called Boxer Indemnity Scholarships (each on one specific subject) to continue advanced education in the US (see *Boxer Indemnity Scholarship Program* listed under Reference.) And it was also a time of forward-looking optimism in the US too, not unlike that underlying the government-funded pure scientific research in Germany of the 18th and 19th century. This pervasive self-confidence of America was also eloquently portrayed by the title of a report written by Vannevar Bush—, the science advisor to both President Franklin D. Roosevelt and President Harry S. Truman—, "***Science:—the Endless Frontiers***."

And for the first time in American history, the US federal government began funding basic scientific research with no strings attached (Atkinson and Blanpied 2008.) The GI Bill of Rights provided financial support for many returning veterans and many of them took up higher education and research in the biomedical fields. The increasing demands for research needs in turn stimulated the growth and creation of a great variety of scientific instruments, chemicals and radio-chemicals that had not existed before—or after. It was in a happy, proud and tolerant atmosphere I received generous supports from all the institutions that I attended in Chicago, in Baltimore and in Philadelphia from 1946 on.

Then suddenly the smooth-sailing American ship hit rock—in the form of the Vietnam War—a war that cost the lives of 60,000 young American and over one million young and not so young Vietnamese, Cambodian and others. As pointed out recently by Michael Keen, this war could very well have been prevented (Keen 2011.) However, we did go into the war and we did not win.

Worse, we also lost the future-oriented optimism on science—somewhere between the late 1960's and the early 1970's. In the dark mood of frustration and despair, which the faltering Vietnam War and the growing skepticism about the benefits of scientific research described in Sect. 1.3.1, cutback on research support followed. Added yet on the woes is that created by the ***peer review system*** installed for government fund allocation and it began to raise its ugly head. For who were they that advised the Chinese Emperor to dismiss the little village boy who told him that the elephant is not like a rope, a wall or a tree trunk but more like a big pig with a long nose. It was the panel of learned (but blind) scholarly peers that could not agree among themselves except in rejecting the little village boy. Who were they that advised Queen Isabelle of Spain to turn down Columbus's plan to reach China by going east? Again it was a panel of three navigator peers. Who were they that had delayed 170 years the acceptance of the Kinetic Theory of Gases and the reality of atoms and molecules? Again it was a collection of scholarly peers. (For yet more of the deadly track of peer review see Ling 1978a, 1998c.)

That I could in fact continue my work for some two more decades was to no small measure the gifts of a few courageous and dedicated scientists-administrators including Dr. Arthur B. Callahan of the Office of Naval Research (ONR) and Dr. Steven Schiaffino of

the National Institute of Health (NIH). But they eventually retired for one reason or another (Ling 2001 p. 367.)

Notwithstanding, I must not overlook my blessing. Thus, before the arrival of still harder times, the ***association-induction theory*** was already well on its feet in theory as well as in experimental verification (Ling 1962, 1965, 1969.) This is why I could announce on a prior page that Mankind now in fact understands what life is in modern physico-chemical terms.

For, in my belief, there cannot be a still more basic and more comprehensive theory in foundation biomedical science than the AI hypothesis. Accordingly, the ***principle of sequential invention*** (Ling 1952) tells us that like the incubation and hatching of a fertile egg, this proven new basic theory would one day begin to generate a world of mankind—benefiting products in areas closest to our long-term wellbeing. To mention just one, rationally designed drugs to combat incurable diseases already in existence and yet to come. *It is the AI Hypothesis that for the first time in history tells us what drugs do to life electronically in modern microscopic physico-chemical terms and its theoretical predictions have already been repeatedly confirmed* (Ling 1962 Chapter 6, pp 107–120; Ling 1984 Figure 7-10 and Figure 7-11 on pp. 204–205; Ling and Fu 1987, 1988.)

But to present the new definition of life and its underlying association-induction hypothesis, I must first update what had happened to biology since mid-19th century with the birth of the new science of cell physiology.

3 The beginning of cell physiology

3.1 The Berlin research university

The great Prussian philosopher, linguist and statesman, Wilhelm von Humboldt (1767–1835) played a key role in introducing the state-supported physiological (and other scientific) research in what came to be known as *research universities* (Humboldt, W. 2008.) Between 1830 and 1850, new physiological laboratories were springing up in the universities all over Germany. Its overall purpose was nothing else than the promotion and nurturing of the search for truth—labeled *Wiesenschaften* or pure science (McClelland 1980, Part III; Ling 2007 p.6.)

Among the research universities established, the first and foremost was the Berlin University. In 1949 what was originally called Berlin University changed its name for the last time to Humboldt Universität in honor of its founder, Wilhelm von Humboldt—and his naturalist brother, Alexander von Humboldt (Humboldt, A. 2010.) To show how well the von Humboldt brothers, but especially Wilhelm von Humboldt deserved this honor and to avoid giving the wrong impression that the former Berlin University only funded physiological research, I cite a list of its illustrious alumni and teachers below (Humboldt University 2010) in addition to those to be mentioned farther beyond in this article:

PHYSICISTS: Max Planck (1858–1947), Albert Einstein (1879–1955), Werner Heisenberg (1901–1976), Max von Laue (1879–1960), Erwin Schrödinger(1887–1961), Max Born (1882–1970), Heinrich Herz (1857–1894), Gustav Herz (1887–1975); **CHEMISTS:** Herman Emil Fischer (1852–1919), Fritz Haber (1868–1934), Jacobus Henricus van't Hoff (1852–1911); **PHYSICIANS:** Rudolf Virchow (1821–1902), Paul Ehrlich (1854–1915), Robert Koch (1843–1910); **PHILOSOPHERS:** Georg Wilhelm

Friedrich Hegel (1770–1831), Johann Gottlieb Fichte (1762–1814), Arthur Schopenhauer (1799–1860), Karl Marx (1818–1883), Friedrich Engels (1820–1895); **STATESMEN:** Otto von Bismark (1815–1898); **POET:** Heinrich Heine (1797–1856); **COMPOSER:** Felix Mendelssohn (1809–1847).

3.2 The cell theory and the membrane (pump) theory

Johannes Müller, a gifted scientist and popular teacher, headed the physiological research institute in the Berlin University. Among the large number of brilliant students he collected around him were the great physiologist-physicist, Herman Helmholtz (1821–1894) and two other members of the "Reductionist Four": Emil Dubois-Reymond (1818–1896) and Ernst Brücke (1812–1892.) The fourth member, Carl Ludwig (1816–1895) was in Leiden (Rothschuh 1973.) As if they were one, the *Reductionist Four* believed that the laws governing the dead world govern the living world too (Rothschuh 1973.) Their overall phenomenal success notwithstanding, the Reductionist Four were not able to resolve the central problem-what life is. The right time for that was yet to come.

First, the basic physical and chemical sciences were themselves still in their early stages of development. Secondly, it was organ physiology that the Reductionist Four were pursuing and organs are not the most basic unit of life. In fact, the cell was already on the way of being recognized as a more basic unit of life by another zoology student of Müller, Theordor Schwann (1819–1882.) In collaboration with botanist, Mathias Schleiden (1804–1881), Schwann introduced the "Cell Theory" in 1839 (Schwann 1839; Schwann 1847; Harris 1999 Chapters 10 and 11.) (For other earlier introductions of the cell theory, see Ling 2007 p. 5; also see Dutrochet 1837.)

Financial support from the government was one contributing factor to Schwann's success in formulating and publishing his "Cell Theory"; the availability of the microscope was another. The deployment of microscopes had also led to the discovery of an even more basic substance of life than the cell. Named ***sarcode*** in 1835 by the French zoologist, Felix Dujardin (1801–1960) who described this substance as a glutinous, translucent and water-insoluble living jelly (Dujardin 1835; Harris 1999 pp. 72–75; Ling 2007 pp. 10–17.)

The critical importance of the sarcode was dramatized by historian Thomas Hall in his treatise on "Life and Matter" (Hall 1969.) In the opening section of Chapter 14, Hall remarked that up to that point, the preceding history he presented in the first thirteen chapters could be regarded as preparation for what would be the subject matter of this Chapter 14. And what is the subject matter or title of that Chapter 14? It is nothing else than *Sarcode*, the living jelly from a protozoon. However, the name sarcode was later replaced by ***protoplasm***—introduced in 1846 by the German botanist, Hugo von Mohl (1805–1872) when he referred to a similar gelatinous substance in plant cells (von Mohl 1846; Harris 1999 p. 72.)

While I have always felt a sense of regret that an earlier name be replaced by a later one, especially since Dujardin went out of his way to give credit to still earlier workers (See Ling 2007 p. 113.) However, there is a defensible justification for this change. The word sarcode came from the Greek word, *sarkodes,* meaning fleshy, which is more appropriate to describe a substance of animal origin and protozoa are tiny animals. To cover both animal and plant materials, protoplasm is a better choice.

Figure 1 shows a viscous stream of plant protoplasm flowing slowly out the cut end of a giant cell of the alga, Nitella (Kuroda 1964.)

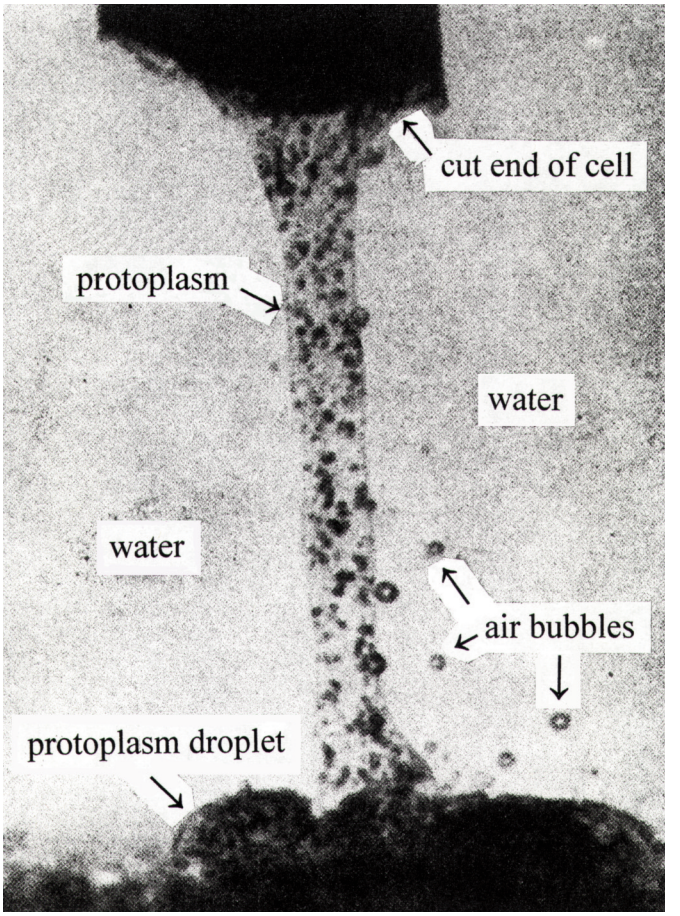

FIGURE 1. Outflow of protoplasm (endoplasm) from the cut end of a giant Nitella cell into a culture medium (labeled as water). The protoplasm collected as a flattened round droplet on the bottom of the cuvette. The photograph was taken 5 minutes after the cut was made. These protoplasmic droplets can survive 10-50 hours in the culture medium containing 80 mM KNO_3, 50 mM NaCl and 4 mM Ca $(NO_3)_2$. (From Kuroda 1964)

The invention of electron microscope and ancillary techniques enabled cytologists to demonstrate that the real cell membrane is only some 100 Angstrom units thick. As such, it is beyond the (ultimate) resolving power of the best light microscopes at 2000 Angstrom units (Davidson 2012.) What this tells us is that Schwann could not have seen and did not see the real cell membrane.

One sequence of Schwann's mistakes began with his erroneous assumption that the large mature plant cell (Figure 2), with its immense watery fluid-filled central vacuole is typical of all plant and animal cells. *He then called the outermost cellulose cell wall* (of a mature plant cell) *plus the layer of cytoplasm lying immediately beneath the cellulose cell wall plus the real cell membrane (together) as the* ***cell membrane*** *(or the* ***cell wall****.)* As a part of his Cell Theory, Schwann then postulated that imbedded in this thick "cell membrane" are microscopic devices (to be called pumps later by others) that control the chemical composition of the fluid inside and outside the cell (Schwann 1839 p. 197; Schwann 1847 p. 199.)

Now, Johannes Müller—who was a full professor as well as the Director of the Physiological Research Institute in the Berlin University—shared a single small room with Schwann; the two also shared the use of a single microscope (Rothschuh 1973.) Both facts testified to the limited financial support even the top ranking Berlin University was

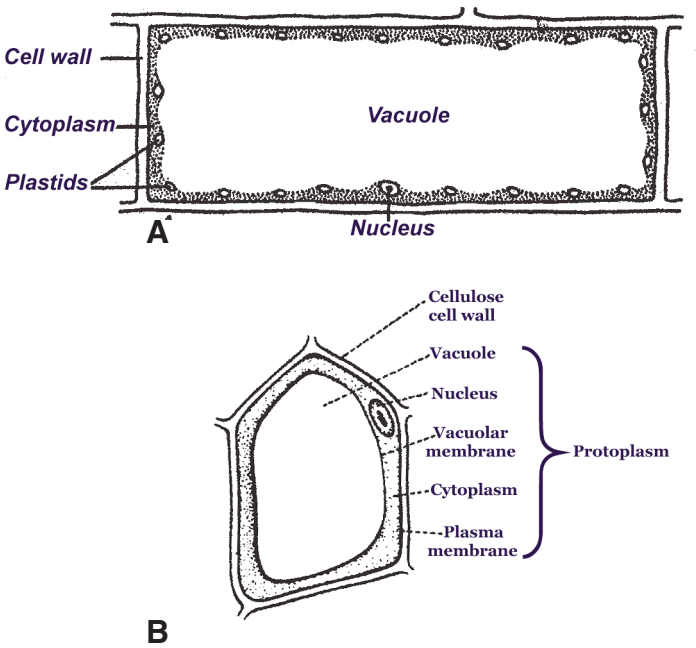

FIGURE 2. Two diagrammatic illustrations of mature plant cells. A. From Heilbrun (1937) who reproduced it from Miller's book of Plant Physiology; B. From Glasstone (1946.)

receiving from the government. Knowing what fantastic achievements these research universities but especially what the Berlin or Humboldt University had accomplished, its limited financial support carries a highly important lesson for future decision-makers on the financial support of science.

It suggests that a modest amount of money used wisely can produce far greater results than a lot of money spent thoughtlessly, an extravagance that not only wastes money but tends to bring into the search for truth, competing alternative motivations like search for power, which money is.

Beyond the small room and the microscope, Müller and Schwann also shared a belief in *vitalism*. Schwann's belief in vitalism was explicitly described in his Magnus Opus (Schwann 1839 p.184; Schwann 1847 (English transl.) pp.186–187.) Since all European universities began as religious institutions to educate future clergies, one is not surprised that even though Schwann's theory contained many serious errors known already at the time, the German textbook producers adopted it without question or dispute (Harris 1999 p. 106.) And as mentioned earlier, Schwann's theory of cells and what came to be known as membrane pumps has remained in textbooks worldwide to this very day, long after it had been disproved (see p. 6.) Again, my conviction is that if we work hard and intelligently on the subject, we will replace it with the right one not too long from now.

3.3 Protoplasmic theory

Ironically, Schwann's membrane-pump notion was far from being universally accepted in Germany at his time. Thus in 1861, thirteen years after Schwann introduced the membrane-

pump concept as a part of the **cell theory,** Max Schultze (1825–1874), Professor of Botany in Bonn, pronounced his *Protoplasmic Doctrine*, according to which, the living cell is a lump of protoplasm with a nucleus but **without a cell membrane** (Schultze 1861; Hall 1951.)

Seven more years later, another historical event took place. Thomas Huxley (1825–1895), described by the straight-shooting Baltimore Sun reporter, H. L. Mencken (1880–1956) as the greatest of English scientists (Mencken 1925)—mesmerized a lay audience in an Edinburgh Presbyterian Church by proclaiming that *protoplasm is the physical basis of life*. The issue of the journal that printed his talk was reprinted an unprecedented seven times (Huxley 1853.) But I must also make clear that it was not bed of roses for Huxley either.

One detractor close by was the Scottish philosopher, James Huchison Stirling (1820–1909.) Stirling pooh-poohed the existence of protoplasm by stating that the same substance could not be at once beef, lobster and the man who eats them (Hall 1969, vol. 2, p. 308.) Another question that could have been asked but was not asked at that time can be put this way: Is the darker nucleus that looks quite different from the surrounding protoplasm also a part of the physical basis of life?" We will return to this subject shortly below on page 18. Answers to Stirling's comment touches on the central role of ATP in life and death, for which the answer will come in the concluding Section 5.1.) For the moment, let us return to the time of Thomas Huxley, his friends and foes.

Far away on the opposite side of the planet, a tavern-owner in Melbourne publicly advertised a well-cooked physical basis of life. Taking offense at this flippancy, Sir Joseph Lockyer came to Huxley's defense in the very first issue of the magazine, **Nature** he had just founded. Lockyer pointed out with vigor and conviction that Huxley took risk in addressing a lay audience not for his own glory but for the common good of all Mankinds (Lockyer 1870.) Yet, by profession, Lockyer was an astronomer, not a cell physiologist. His vigorous defense of Huxley bespoke of a time that leading scientists took themselves seriously of their global responsibility in educating and caring for the future inhabitants of this small planet we share.

In the wake of the historic contributions of Max Schultze and Thomas Huxley, the protoplasmic approach flourished in the remaining decades of the 19th century. Indeed, as late as 1908, William A. Locy, Professor of Biology at the Northwestern University of Illinois in the United States wrote these words in his "*Biology and its Makers*": "All future progress will be made by studying this living substance (protoplasm)—the seat of vital activity. This was the beginning of modern biology." (Locy 1908.)

Locy too would have turned in his grave if someone were to tell him what happened to protoplasm after his optimistic forecast. It was quietly removed from textbooks and what we teach to the younger generation, ostensibly as a new addition to the list of once prominent but ***genuinely*** erroneous concepts—like Lavoisier's *caloric* and Georg Stahl's *phlogiston*.

Thus, advances in microscopy and related fixing and staining techniques have revealed in cells more and more sub-cellular structures and organelles. Like the nucleus, they too looked different from what Dujardin and von Mohl once described as sarcode or protoplasm. Summarizing this anticlimactic ending, the Encyclopedia Britanica Online stated in 2011: "*As the cell has become fractionated into its component parts, protoplasm, a term no longer has meaning.*" In truth, Encyclopedia Britanica Online and like-minded instigators of the disappearing protoplasm are all wrong.

And, they are not just wrong but wrong in a strange way—repeating an error made in an earlier attempt to erase the concept of protoplasm in the 1930's. In both cases, what were disproved were not the existence of protoplasm but two sets of mistaken theoretical concepts on the nature of protoplasm. (Details of the earlier case will be found on page 30 below.)

In this, the second suggested trashing, the wrong theoretical concept was not even explicitly pronounced but was assumed to be true by others. That concept is, Dujardin and von Mohl had considered all protoplasms to be in texture and appearance like the translucent, glutinous, water-insoluble living jelly that flows out a broken protozoa or large plant cell as shown in Figure 1. Repeating what was mentioned briefly above, I shall discuss the details of the wrong theory that led to the first false abandonment of the concept of protoplasm in the section on cell-water in Section 4.3 below.

Happily, in both cases, the AI Hypothesis was able to come to the rescue (see below.) As a result, the abandoned protoplasm was resurrected again and Locy's optimistic outlook re-established a second time.

4 The association-induction hypothesis

It took me all told 15 years to complete the new unifying theory of the living cell called the **association-induction hypothesis** or AI Hypothesis for short and AIH for shorter. The theory arrived in three parts. The first part is an embryonic version of the AI Hypothesis known as Ling's Fixed Charge Hypothesis centered on the selective K^+ accumulation in living ells (Ling 1952.) The second part marked the development of the AI hypothesis proper (Ling 1962.) The third part was first introduced under the name, the Polarized Multilayer (PM) theory of cell water and model systems (Ling 1965), only to be replaced later by the name, the Polarized-Oriented Multilayer (POM) theory of cell water and model systems (Ling 2003.) However, it was the introduction of the PM theory in 1965 that completed the presentation of the unifying theory.

In order to present the answer in a readily understandable manner to the question, What Is Life?, the relevant parts of the entire AI Hypothesis will be reviewed beforehand. But to make the presentation of the relevant parts of the AI Hypothesis themselves easily readable, I will present its two intellectual foundations or supporting pillars first—as I had done once in 1962 and I am doing it here once again.

4.1 Foundation pillars

The twin pillars on which the ***association-induction hypothesis*** is built are (1) the science (physics) of Statistical Mechanics and (2) the (correct part of) protein and polypeptide chemistry and physics. Accordingly, the first chapter of the monograph "*A Physical Theory of the Living State*" presents the fundamentals of *Statistical Mechanics*, which as pointed out earlier, the ill-fated Austrian mathematician-physicist, Ludwig Boltzmann invented almost single-handedly. In the 7th Chapter of the same monograph, I also introduced a new theory of proteins founded on the basic knowledge on proteins, which the great German chemist (Herman) Emil Fischer uncovered largely in the late 19th and early 20th century (Fischer 1906.)

4.1.1 Basic protein chemistry

Long before Locy wrote his prophetic comment on the living matter, Emil Fisher (1852-1919) had worked out the complex structure of proteins, which form the distinctive components of all living matter. Fischer also introduced the name, ***polypeptide***, which is a unique kind of organic macromolecules made only by living organisms (including synthetic organic chemists and engineers.) (Fischer 1906)

The chemical reaction that produces a polypeptide from free individual α-amino acids (or simply amino acids) is illustrated as follows:

$$NH_2\text{-}CH\text{-}(R_i)\text{-}COOH + NH_2\text{-}CH\text{-}(R_i)\text{-}COOH + NH_2\text{-}CH\text{ -}(R_i)\text{-}COOH \rightarrow$$

$$NH_2\text{-}CH\text{ -}(R_i)\text{-}CONH\text{-}CH\text{-}(R_i)\text{-}CONH\text{-}CH\text{-}(R_i)\text{-}COOH + 2H_2O. \quad (1)$$

Note that two *peptide linkages* (CONH) are formed from three free amino acids. Between each pair of amino acids that form a peptide linkage, two hydrogen atoms and one oxygen atom are lost in the form of two water molecules. The remainder of each amino acid in the protein formed is called an *amino acid residue*.

As a rule, each amino acid residue endows the protein a different side chain shown as R_i in the formula for the amino acids given above. As an example, R_i is a single H atom for the amino acid residue glycine; it is a methyl group for the alanine residue. R_i for aspartic acid residue is CH_2COOH, carrying at its end a β-carboxyl group. R_i for glutamic acid residue is CH_2CH_2COOH, carrying at its end a γ-carboxyl group. R_i for lysine residue is $(NH_2)CH_2\ CH_2\ CH_2$, carrying at its end an ε-amino group. R_i for arginine is $NH_2C(NH)_2\ CH_2CH_2CH_2$, carrying at its end a guanidyl group. In a neutral aqueous medium, the β-, and γ-carboxyl group are ionized and each carries a net negative charge and thus functioning as a mono-valent anion. In contrast, the ε-amino group and the guanidyl group each carries a net positive charge and as such, it functions as a mono-valent cation. Immobilized by their anchorage onto the lengthy protein chains, these anions and cations are referred to respectively as *fixed anions* and *fixed cations*. When a fixed cation joins a fixed anion and forms an electrostatic bond, a ***salt linkage*** is formed (Speakman and Hirst 1931.) Salt linkages, like its counterpart ***α-helical H bonds***, determine mostly the folding patterns known respectively as the tertiary and secondary structures of a protein. (For strong evidence of the key role of salt-linkages in the maintenance of the tertiary structure—contrary to belief of some protein chemists—, see p. 24 in Sect. 4.2 below.)

A distinctive feature of all life forms is its pervasive *connectedness*. The underlying long-distance information and energy transfer has been compared to that of a chain of falling dominos, (which goes only one way and thus irreversible) or a chain of tethered frictionless see-saws. In the latter case, a tiny perturbation (like that brought about by a curious visiting mouse) at one of the terminal seats of the chain can cause the entire chain to flip from one stable conformation to the only other alternative stable conformation as illustrated in Figure 3. (See also Ling 1962 pp. 145–146.)

The basic mechanism in both the falling domino chain and the chain of frictionless seasaws is mechanical and relies on gravity. In contrast, the long distance information and energy transfer in proteins is, according to the AI Hypothesis, fundamentally electronic as

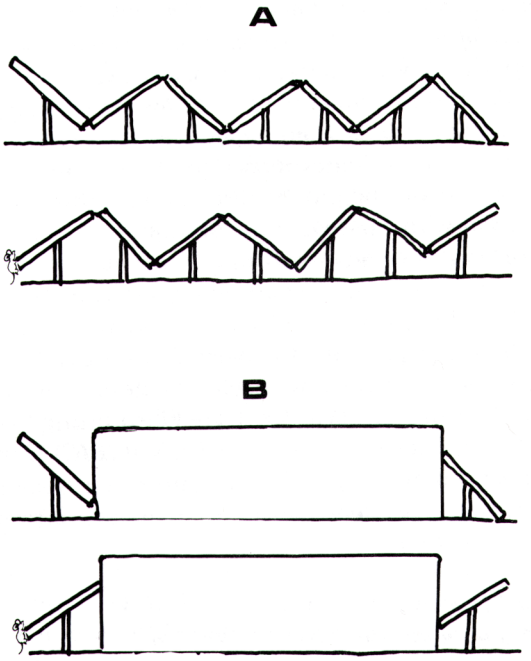

FIGURE 3. Mouse and Seesaw Chain Model. Figure A demonstrates how extensive changes over long distance can be achieved in a delicately balanced system in response to a minute energy input at a suitable site. Partial perception of the system (Figure B) produces the impression of a "magical happening". (From Ling 1992)

the word, *induction* indicates (Ling 1962 pp. xxxii–xxxvi.) And yet, protein chains are not electronic conductors. Quite the contrary, silk fibers, comprising a single pure protein, called silk fibroin, have been traditionally used to suspend and insulate pith balls in early demonstration of electric attraction and repulsion.

While all proteins share *connectedness*, each protein is distinct from all other proteins. In their diversity, proteins resemble words in some languages. Thus, the diversity of words in the English language comes from linear arrays of assortments of the 26 letters of the alphabet. The diversity of a protein, on the other hand, comes from a linear array of assortments of some 20 amino acid residues. That the words, *eat* and *ate* are different in meaning is entirely arbitrary. That the small polypeptide A (glu-gly-lys) differs from the small polypeptide B (glu-lys-gly) has an ***electronic*** basis.

Thus, the γ-carboxyl group carried on the side chain of the glutamic acid residue (glu) in polypeptide B is a stronger acid than the γ-carboxyl group in the polypeptide A. This difference arises from a combination of two causes. First, the γ-carboxyl group is closer to the positively charged ε-amino group of the lysine residue (lys) in polypeptide B than in peptide A. Second, the neutralizing influence produced by the positively charged ε-amino group diminishes with the distance separating the *effector* group and the *target* group of a large molecule. Weakening of the fixed anion for the positively charged hydronium ion, H^+, means that more free H^+ exists in the medium and that, in turn, reveals that its source

acid is a stronger acid—in a sequence of elementary events that will be further elaborated upon below.

An electronic effect that is transmitted through intervening space is called a direct or D-effect. On the other hand, electronic effect transmitted through the intervening linked atoms is called an inductive or I-effect. I-effect and D-effect acting together are known as F-effect (Hermans 1954.) Next, we discuss one kind of the *target group* of these electronic effects in the form of the carboxyl group of acetic acid or HAc.

In neutral water, part of the acetic acid dissociates into a positively charged hydronium cation, H^+ and a negatively charged anion, Ac^-:

$$HAc \leftrightarrow H^+ + Ac^-. \tag{2}$$

When equilibrium is reached, there will be a quantitative relationship among the concentrations of the individual ions and molecule as shown next.

$$K_a = ([Ac^-] [H^+]) / [HAc] , \tag{3}$$

where $[Ac^-]$, $[H^+]$ and [HAc] are the molar concentration of the dissociated acetate anion, the dissociated hydronium ion and the undissociated acetic acid respectively and K_a *is* the *acid dissociation constant*. Shown in the following Equation 4 is the pK_a of this acid (HA) equal to the negative logarithm of the acid dissociation constant, K_a to the base 10:

$$pK_a = -\log_{10} K_a . \tag{4}$$

Having made clear what pK_a stands for, we proceed to examine how it is determined by the molecular structure of the acid. Consider as an example acetic acid, CH_3COOH. It is an acid that makes our salad pleasantly sour but not too sour. Accordingly, it is a weak acid with a high pK_a equal to 4.756. In contrast, trichloroacetic acid (TCA), CCl_3COOH is a chemical agent we use in the laboratory to denature proteins and make them insoluble in water and thus easily separable from other components of an aqueous mixture. Though sharing the same carboxyl group with acetic acid, trichloroacetic acid or TCA is a very strong acid. Its pK_a is below unity at 0.66.

The profound difference in pK_a of acetic acid and TCA tells us that the negatively charged oxygen atom in the dissociated carboxyl group attracts and thus holds onto the hydronium ion much more tightly in acetic acid than in TCA. This difference in turn reflects the profoundly different impact the attachment (onto the α-carbon atom of the acid) of a chlorine atom has than that of a hydrogen atom on the pK_a of the adjacent carboxyl group. The reason is as follows.

The atomic weight of the chlorine atom is 35.5 and its atomic number is 17. This number indicates that there are 17 protons in the nucleus of each chlorine atom, whereas there is only one proton in the nucleus of a hydrogen atom. Since each proton carries a positive charge, the orbiting negatively charged electrons in a chlorine atom are much more strongly attracted to the nucleus of a chlorine atom than the orbiting electron in a hydrogen atom is attracted by the single proton in the nucleus of a hydrogen atom. When three H atoms on the α-carbon atom of acetic acid are replaced by three chlorine atoms, electrons in the vicinity are drawn toward the chlorine atoms and their aggregate impact is

passed on to reach the distant singly charged oxygen atom of the carboxyl group. The net result is a reduction of the effective negative charge of the oxygen atom and a lowering of the attraction between that oxygen atom and (free) hydronium ion, thereby making TCA a much stronger acid than acetic acid. Indeed, it is precisely with this example of acidity change from acetic acid to TCA that G. N. Lewis introduced his Induction Theory in 1923 (Lewis 1923.)

The development of Quantum Mechanics revolutionized physics and chemistry. In 1933 James and Coolidge using elaborate wave mechanical methods, were able to derive quantitative attributes of the hydrogen molecule with accuracy to the sixth decimal place (James and Coolidge 1933.) For a while, many felt that it was a matter of time before physicists would be able to solve all chemists's problems with immense accuracy. However, this optimistic outlook was less than realistic. Indeed, in the ensuing years to this very day, neither James, nor Coolidge nor anyone else has succeeded in explaining in wave-mechanical or other terms, the striking difference in the pK_a of acetic acid and TCA. Indeed, we are back at the roots again. In dealing with isolated single bodies like a distant star or even a single hydrogen molecule, physicists can do marvels. Again in dealing with vast number of items, statistical mechanics can provide equally accurate computations. It is in dealing with entities somewhere between, like an acetic acid, that it is hopeless to try to achieve the kind of accuracy physicists are used to in their chosen models of utter simplicity.

Lewis's Induction theory and its follow-ups have provided the backbone of theoretical organic chemistry in the hands of Hammett (1940), Branch and Calvin (1941), Ingold (1953) and Taft and Lewis (1958.) Both Hammett and Taft have provided useful empirical constants for chemical groups that can be used to predict quantitative data of new chemicals. However, it was Chiang and Tai who have profoundly improved the theory by liberating it from the restriction imposed by the limited empirical constants available. They did it by introducing a method of calculating what they call ***inductive indices***—from known and accessible parameters of molecular structure, atomic electronegativity and bond length (Chiang and Tai 1963. For brief description of key ideas, see Ling 1984 pp. 185–188; Ling 1992 p. 113.)

4.1.2 Statistical Mechanics

When you flip a coin, there is no way to foretell whether tail or head would come up. However, if you flip the coin a million times, you can foretell with great accuracy that half would be heads, the other half would be tails. The mathematical science that deals with laws governing large numbers is, of course, *statistics*.

In the 17th century, Boyle's Law was discovered. It tells us that the volume (v) of a body of gas is inversely proportional to its pressure (p) so that the product pv is a constant. However, the foundation of this law was entirely empirical. For that reason, it could not explain why pressure is exerted on the container wall in all directions. In 1738, Dutch-Swiss mathematician Daniel Bernoulli (1700–1782) first introduced the ***Kinetic Theory of Gases***, in which air or gas represents a vast number of rapidly moving, extremely small corpuscles or gas molecules, their bombardment on the container wall producing the all-directional pressure. However, Bernoulli's theory was rejected. So were three other independent physicists each presenting an improving but basically similar theory. They

include English physicist, John Herapath and a Scottish scientist employed in India, John James Waterston and the Austrian mathematician-physicist, Ludwig Boltzmann. All told it took one hundred and seventy (170) years before physicists finally accepted atoms (and molecules) as real—unbelievable as it appears to me today (Brush, S.G. 2003.) Of more direct relevance to my work, however, was the fact that Boltzmann did not just present the most advanced version of the kinetic theory of gases. He also introduced the new science of ***statistical mechanics***, *which explains macroscopic natural phenomena in terms of the vast number of microscopic atoms and molecules in precise quantitative terms* (Cohen 1997.)

Both the membrane (pump) theory (of Schleiden and Schwann) and the *original* protoplasmic theory (of Dujardin, von Mohl, Dutrochet and Huxley) were attempts to explain natural phenomena in terms of ***macroscopic*** concepts like membranes, semipermeability for the membrane (pump) theory and glutinous, diaphanous and water-insoluble traits for the (original) protoplasmic theory. Like the Kinetic Theory of Gases, the association induction hypothesis is a theory that attempts to explain macroscopic (living) phenomena in terms of vast numbers of **microscopic** molecules, atoms, ions and electrons—as made clear in the title of this communication.

As an example, in the immediately following section is a very useful statistical mechanical formulation on the distribution of molecules in space called the *Boltzmann distribution law*. (See *Luke, BT & Assoc*. in the Reference List.) With its help, one can, for example, determine the density of dust particles in the atmosphere in locations not too far from the surface of the earth. More precisely, the kinetic energy of the dust particles tends to move the dust particles farther away from the earth while gravity restrains that movement. The relative density of dust particles is then expressed as an exponential function of the ratio of gravitational attraction energy divided by the average kinetic energy equal to kT, where T is the absolute temperature and k is the Boltzmann constant. A similar formula will be used in the new theory of selective accumulation of one species of cation, K^+ over another one, Na^+—in the embryonic version of the AIH introduced in 1952 and known as Ling's Fixed Charge Hypothesis (Ling 1952.)

In pages immediately following, I shall begin with a simple account of the key features of Ling's Fixed Charge Hypothesis. It will be followed by a simple account of the Polarized-Oriented Multilayer theory of cell water (Ling 1965.) After that, there will be a brief presentation of the association-induction hypothesis proper (Ling 1962.)

4.2 Ling's fixed charge hypothesis

Some time in the year 1950 I was, as usual, reading and thinking in the Welsh Library of the Johns Hopkins Medical School. Suddenly, a new idea struck my mind. It was a theoretical mechanism for the selective accumulation of K^+ over Na^+ in living cells (and in inanimate model systems) that would not require a continual supply of energy as in the membrane (pump) theory. For a moment at least, I was ecstatic.

The new idea arose from a synthesis of several basic facts of physics. First, properties of matter can be roughly sorted into two categories. Long-range attributes include sight and sound that can be perceived at different distances away from their origin. Short-range attributes include textures and taste of an object, which can be perceived only by direct contact. Now, the long-range attributes of the pair of mono-valent cations, K^+ and Na^+ are indistinguishable. In contrast, the short-range attributes of size are different in these two ions with a twist. The (naked) K^+ is larger than the (naked) Na^+. However, when brought

into contact with water, the smaller (naked) Na^+ takes on a more or less permanent coat of hydration water thicker than that taken up by the larger (naked) K^+. As a result, the hydrated Na^+ is substantially larger than the hydrated K^+. However, to experience this size difference, these cations have to be brought into close contact with a sensing device such as a set of fixed negative charges or fixed anions.

Fixed charges have been on the menu for a long time. Only those fixed charges widely considered up to that time are ***fully dissociated*** from their oppositely-charged free ionic partners or counter-ions (For illustrations, see Figures 2 and 3 on p.6 of Ling 2005.) This concept of full dissociation between the fixed charges and their counter-ions reflects the widely adopted principle of full ionic dissociation in aqueous media, often associated with names of scientists including physical chemists Jacobus van't Hoff, Wilhelm Ostwald, Sven Arrhenius and physicist, Peter Debye. It was therefore against the popular belief that I introduced in 1952 the new idea of enhanced ionic association when one of the reacting charges or ion is fixed in space in these words (Ling 1952 p. 769):

> " (i) The force of attraction between ions of opposite signs in solution is opposed by the kinetic energy of the ions themselves. If one of the ions is rigidly fixed, half of this energy is abolished, so that the ions stay on the average closer together than when the kinetic energy of both reacting charged particles is made negligible, as for example in the macroscopic model of oppositely charged pith balls.
>
> (ii) Fixation allows the close juxtaposition of a number of similarly charged ions, for the repulsive forces between them are less strong than the covalent bonds of fixation. The individual fields thus overlap, and sum with respect to the force exerted collectively upon a free ion of opposite sign."

These simple statements referred to, as "*the Principle of Enhanced Ionic Association by Site Fixation*" was valid then, as it is valid today. And for what we try to do here, it was adequate then, as it is adequate now.

Notwithstanding, fifty-three (53) years after the 1952 publication, I further developed and presented the more detailed underlying theory in a paper entitled: "An Updated and Further Developed Theory and Evidence for the Close-contact, One-on-one Association of Nearly All Cell K^+ with β- and γ-Carboxyl Groups of Intracellular Proteins" (Ling 2005.) For those interested in this more advanced version, he or she can download a free pdf version online. (For direction, see Ling 2005 in Ref. List)

In addition, I want to add that the misguided adoption of the ionic dissociation theory described in Ling (2005, Appendix A on pp. 50–53) also made many protein chemists reluctant to recognize even today the idea that salt linkages formed between pairs of fixed cations and fixed anions are the most important part of the tertiary structure of many folded protein as I had suggested in 1962 (Ling 1962 p. 249) and repeated again and again ever since.

Referring to it as the "salt-linkage hypothesis", Ling and Zhang then published in 1984 a set of strong experimental evidence of the dominant role of the salt linkages in the maintenance of what has been referred to as the tertiary structure of many globular proteins (Ling and Zhang 1984; Ling 1992 p. 44; Ling 2001 p. 55, 238, 323.)

Having established the high propensity of the K^+ (or Na^+) to associate with the fixed negative charges, I then pointed out in the same 1952 article that in living cells, most of the fixed negative sites exist in the form of β- and γ-carboxyl groups carried respectively on the aspartic-acid and glutamic-acid residues of intracellular proteins. In frog muscle, I showed that the protein myosin alone carries enough β- and γ-carboxyl groups to associate

with all the K^+ and Na^+ found in the cell. The next step was to invent a mechanism of selective accumulation of K^+ over Na^+—as found in most living cells that have been carefully studied. And as made clear on a preceding page, I succeeded.

The Coulomb Law dictates that the electrostatic attraction between a positive electric charge and a negative electric charge varies directly with the product of the sign and magnitudes of the two charges and inversely with the square of the distance between the charges. However, when the ions are in water, the interaction is severely reduced by a familiar number called the *dielectric constant*, usually given a value of 81. But when the ionic interaction takes place at very close range in water, the phenomenon of *dielectric saturation* kicks in, lowering sharply the value of the dielectric constant according to the distance of separation as shown in the inset of Figure 4 (Hückel 1925; Debye and Pauling 1925; Hasted *et al* 1948; Grahame 1950; Ling 1952.) With these background information on hand, I then presented a mechanism of selective K^+ accumulation over Na^+ in living cells and model systems.

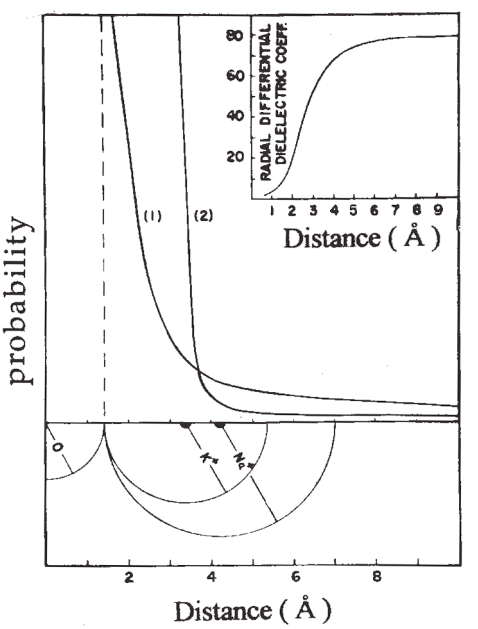

FIGURE 4. A theoretical model for the selective accumulation of K^+ over Na^+ in a fixed oxyacid site presented in 1952 as a part of Ling's Fixed Charge Hypothesis (LFCH). The computation takes into account the decrease in the dielectric constant of water (referred to in the Inset as "radial differential dielectric constant") when approaching a fixed ion as illustrated in the inset. Theoretical curve (2) shows the probability of finding a mono-valent cation (e.g., K^+, Na^+) associated with the fixed oxyacid anion—partially represented at the extreme left of bottom section of the figure—at a distance away from the center of the oxygen atom of the oxyacid group indicted on the abscissa in Angstrom units. Note that only the hydrated K^+ with its smaller radius (shown in the bottom figure) can enter the "shell of high probability of association" around the negatively charged oxygen atom of the oxyacid group and becomes preferentially adsorbed over the larger hydrated Na^+, the center of which stays largely out of the shell of high probability) also shown in the bottom part of the figure. (From Ling 1952)

Let us begin by focusing our attention on a ***single*** anionic oxygen atom of an oxyacid group like a β-, or γ-carboxyl group—among the vast number of similar anionic oxygen atoms inside a single muscle cell. The impact of dielectric saturation as illustrated in the inset of Figure 4 is to create around the anionic oxygen atom a shell of elevated probability for finding a free (mono-valent) cation. However, the main picture of Figure 4 shows that only the hydrated K^+ is small enough to enter into the space or "shell" of high probability and become accumulated therein. On the other hand, the larger hydrated Na^+ could not come that close to the center of the oxygen atom of the oxyacid group and must settle for a different space or "shell" of lower probability farther away. Since only one mono-valent cation accompanies each fixed mono-valent oxyacid anion, the larger hydrated Na^+ becomes thus selectively excluded by the competing K^+. This theoretical model yields a 7 to 1 preference of the smaller hydrated K^+ over the larger hydrated Na^+—, which is lower than found in many types of living cells, which could be as high as 40 (see below)— but in the right direction.

When I first discovered this new idea I was very excited and thus in a mood probably not unlike that of Archimedes (287–212 BC), when he found a new way to estimate the volume of irregularly shaped golden crown of unknown purity. But I did not go on the street naked shouting "Eureka!"—as legend tells us that our illustrious predecessor did. Yet I must have told a few friends excitedly about it and some of them remembered.

One early afternoon I was walking on the boardwalk of the Johns Hopkins Medical School in the general direction of the Welsh Library, when I saw an overflowing crowd at the entrance to the main (sloping) auditorium. Just after I had found out that it was Professor A. B. Hastings from Yale University giving a talk on his expertise subject, K^+ in living systems, I heard a cry in the audience "Is Dr. Ling here?"

For a moment I hesitated. But with encouragement from the audience, I ended up standing on the podium and trying to draw on the blackboard a picture like that shown in Figure 4—to illustrate how selective K^+ accumulation over Na^+ could be achieved without continual energy consumption. After I finished, Prof. Hastings, the honored guest speaker, walked over to me and shook my hand, saying at the same time that all his life he has suspected the selective K^+ accumulation in the living cell had something to do with the hydrated ionic diameters. And then he added: "Now you got it."

In years following, recollection of this moment keeps on returning to my mind. It is great man like Prof. A. B. Hastings that has made scientific research uniquely rewarding. And for this reason, I have been telling this story every time I had a chance and will continue to do so in the future—if only to show the young generation that the road to scientific discoveries is not all paved with jagged and dangerous rocks. It also has its softer and kinder moments.

In the six decades following the introduction of Ling's fixed charge hypothesis (FCH), many studies have been carried out in our laboratory and elsewhere. And they have consistently affirmed experimentally the validity of the theory that almost all K^+ in frog muscle cells are indeed (electrostatically) adsorbed one-on-one, in close contact on the β-, and γ-carboxyl groups of myosin and other cell proteins. The following are examples:

(1) According to Ling's FCH (and its later version the association-induction hypothesis [AIH]) virtually all cell K^+ compete for the same binding sites and the effectiveness of a specific kind of mono-valent cation in displacing other ions from these binding sites should vary with their respective short-range attributes. In contrast, if the K^+ is free as in the membrane or membrane (pump) theory, all mono-valent cations should behave and act entirely alike—qualitatively and quantitatively. Using radioactive

isotope-labeled K^+ and other alkali- metal ions, Ling and Ochsenfeld (1966) showed that the effectiveness of the same concentration of one hydrated ion in displacing two different mono-valent ions differs sharply.

(2) According to Ling's FCH (and its later version the AIH), in voluntary muscle cells, much of its cell K^+ are engaged in one-on-one, close contact adsorption on the bountiful β-, and γ-carboxyl groups of myosin found on the two edges of the A-bands (Engelmann 1873; Hanson and Huxley 1953.) The FCH (and AIH) predicts that cell K^+ or its surrogates should be found also on the two edges of the A-bands. In contrast, according to the membrane theory or membrane (pump) theory, K^+ and its strongly adsorbed surrogate mono-valent cations should be found wherever there is (cell) water. And accordingly, a more or less even distribution of K^+ throughout the cell is the expectation. However, since the I-band has a somewhat higher water content than the A band (Huxley and Niedergerke 1958), the even density of surrogate ions in the I bands should be somewhat higher than in the A bands.

Using a variety of sophisticated technologies, Ludwig Edelmann from Germany has developed one innovative technique after another, steadily and consistently refuting the prediction of the membrane or membrane (pump) theory. At the same time, these studies have provided some of the most convincing visual proofs that the surrogates of K^+ like Cs^+ and thalium ion (Tl^+) are like K^+ engaged in one-on-one, close-contact adsorption on the two edges of the A-bands in living (fully-hydrated) frog muscle cells as predicted in Ling's FCH (Edelmann 1988; Edelmann 1989; for review, see Ling 1992; see also Ling and Ochsenfeld 1991.)

Figure 5 from Edelmann (2001) provides a shining example. In (a) a 0.2 μ (or 200 nm)-thick section of a freeze-dried and embedded frog muscle was exposed to an aqueous solution of 100 mM LiCl and 10 mM CsCl. Viewed under an electron microscope, it shows that the electron-dense Cs^+ ions (atomic weight 132.9) adsorbed onto the two edges of the (dark) A bands (and the Z-lines in the middle of the (light) I bands. In (b) similar exposure to the Li-Cs solution of a muscle killed by prior exposure to glutaraldehyde produced no localized adsorption of Cs^+ as shown in (a).

4.3 The polarized-oriented-multilayer theory of cell water

As an integral part of the association-induction (AI) hypothesis, the polarized-oriented multilayer (POM) theory was first presented in 1965 at a symposium under a somewhat different title: polarized multilayer (PM) theory (Ling 1965)—three years after the publication of the association-induction hypothesis proper (Ling 1962.) The title of the Symposium was "*Forms of Water in Biological Systems*." It was sponsored conjointly by the New York Academy of Science and the Office of Naval Research.

4.3.1 Exclusion of Na^+ /other large solutes and other physical-chemical attributes of polarized-oriented water

A main point made in this 1965 presentation is that *all or virtually all the water in the living cell assumes the dynamic structure of polarized-oriented multilayers*. Figure 6 is a reproduction of the key original figure I presented at that conference demonstrating the suggested mechanism.

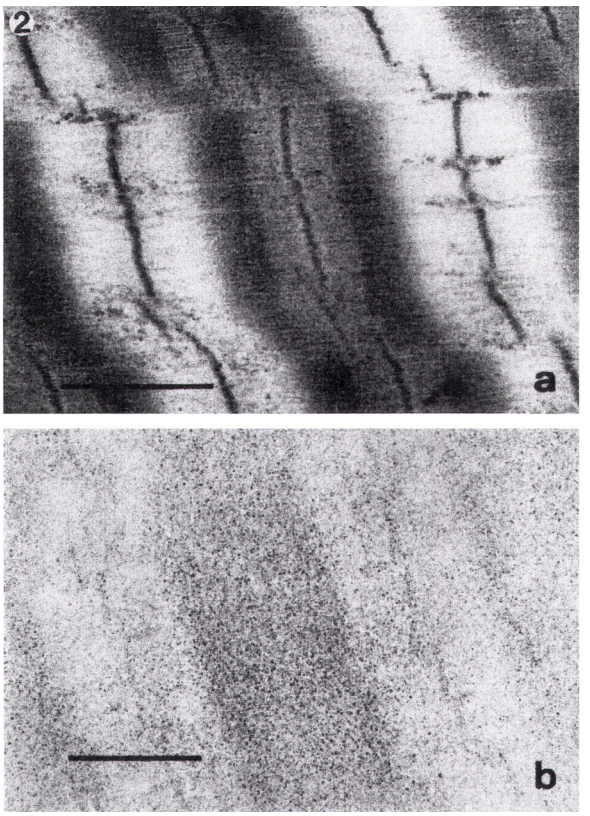

FIGURE 5. 0.2 μm-thick section of frog sartorius muscle stained with a solution containing 100 mM LiCl and 10 mM CsCl as described by Edelmann (1984.) (a) Freeze-dried and embedded sections without chemical fixation. (b) Glutaraldehyde fixed (and thus killed) and conventionally embedded muscle. Bars: 1 μm. (Edelmann 1988, 2001)

In a preceding section, I have already described the one-on-one, close contact association of virtually all the intracellular K^+ on the β-, and γ-carboxyl groups. Since water, K^+ and proteins make up the bulk of the osmotically active substances of all living cells, the association aspect of the AI Hypothesis in this brief narrative is now complete.

The second main point made in the 1965 presentation is that water assuming such a dynamic structure excludes (incompletely by a small margin) larger solutes like sucrose and hydrated Na^+. Hence what is known as the "**size rule**": the larger the solute molecule, the lower is its equilibrium concentration in a polarized, oriented cell (or model) water (Ling 1993; Ling and Hu 1988; Ling, Niu and Ochsenfeld 1993.)

There are two basic mechanisms for this (incomplete) exclusion of larger solutes: an *energetic* mechanism and an *entropic* mechanism. We will begin with the energetic mechanism.

Since the water-to-water interaction energy is higher in the dynamically structured cell water than in the bathing normal liquid water, it would need to spend extra energy in excavating a hole in the cell water to accommodate sucrose or hydrated Na^+ than the energy recovered from filling the holes left behind by these solutes in the surrounding normal water. And the net energy difference in energy expenditure and recovery is the larger, the larger the solute molecule or hydrated ion involved. The Boltzmann distribution law then dictates a lower concentration for the larger solute in the dynamically structured water.

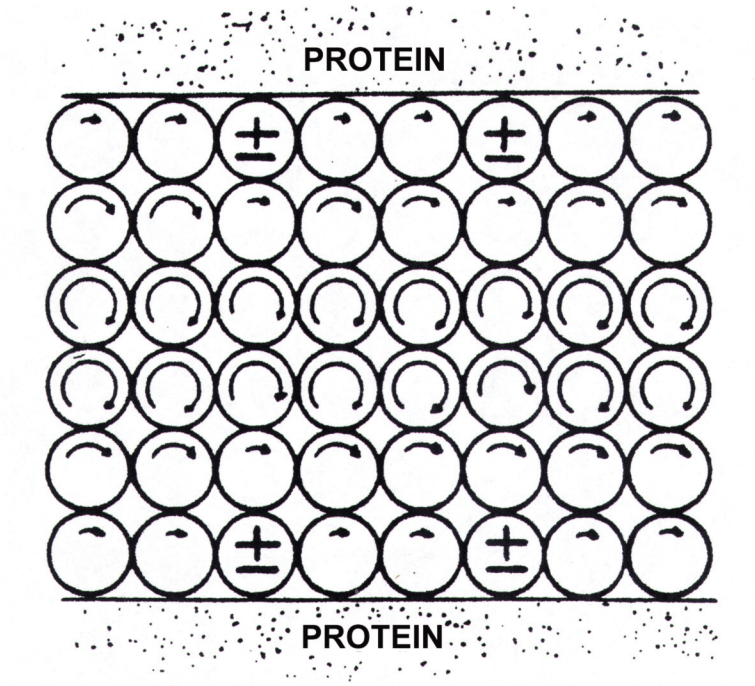

FIGURE 6. The original illustration of the polarized (oriented) multilayer theory of cell water, in which each water molecule is represented as a circle containing a curved arrow. The length of the arrow indicates the assumed degree of motional freedom. Later work shows that the degree of motional restriction is much more uniform rather than showing a steep gradient as suggested in this original illustration. (From Ling 1965)

The excess water-to-water interaction energy is given the name, *exclusion intensity* and represented by the symbol, U_{vp}. We soon found out that this parameter has at first sight, an unusual feature. That is, the absolute magnitude of the excess energy in cell or model water capable of producing a striking size-dependent solute exclusion is very small—when compared to the total water-to-water interaction energy. The outcome is the consequence of a balancing act—like that operating in an elevator or an analytical balance. As an example, the *exclusion intensity (U_{vp}) is only 126 cal/mole in the dynamically structured bulk phase water of living frog muscle cells*—and thus orders of magnitudes lower than the vaporization energy of water equal to about 10,000 cal/mole. U_{vp} of all the various *extrovert* model systems that we had studied and had demonstrated size-sensitive solute exclusion are even lower than that found in frog muscle cells (See Ling, Niu and Ochsenfeld 1993.)

The *entropic* mechanism of solute exclusion also shows a variation with the size of the excluded solute. Here too, the larger the solute molecule (or hydrated ion), the larger the degree of exclusion. First, the larger molecules are more likely to have more varieties of motional freedoms. As an example, a simple mono-atomic solute has only one degree of motional freedom, namely, the *translational*. Big and complex molecules like sucrose, on the other hand, have one to more *rotational* motional freedom in addition to the translational freedom. Furthermore, these rotational motions are more likely to be restricted by the less mobile polarized-oriented bulk-phase water molecules, further reducing their entropy.

Beyond the bulk-phase energetic and entropic mechanism described above, there is a third factor that may come into play and it concerns the surface of the excluded solute (Ling 1993.) Thus, the surface structure of some molecules or assemblies of molecules may fit the surrounding dynamic water structure and thus creates a favorable energy for the retention of that molecule or assembly of molecules in the cell (or model) water. Urea, ethylene glycol belong to this category and like ethylene glycol, so are a number of the so-called *cryoprotective agents* that prevent living cells from being damaged when cooled to and stored in liquid nitrogen at a temperature of −180°C as given by the authors (Luyet and Hartung 1941; Polge, Smith and Parkes 1949; Rall 1987) or even lower at close to absolute zero (Ling and Ochsenfeld 2008.)

In one extensive study reported by Ling and Ochsenfeld (1989), it was shown that the same solution containing a high concentration of polyvinylpyrrolidone or PVP (which polarizes and orients the bulk phase water) partially excludes sucrose but at the same time demonstrates an equal distribution for urea—in full accord with the PM theory of cell and model water solvency summarized above. This demonstration of equal solvency for some solutes and unequal solvency for other solutes refutes the first rejection of the concept of protoplasm for a wrong reason, namely bound or non-solvent water is present in all protoplasm and bound or non-solvent water excludes 100% all solutes big, small or in-between. A.V. Hill's discovery that urea distributes equally between muscle cell water and external medium, was seen widely then as an incisive disproof of the concept of bound water and that of protoplasm defined then by its possession of bound water (Hill 1930; Hill and Kupalov 1930; Blanchard 1940.) Hill's conclusion was wrong because he only disproved an erroneous theory.

We now introduce a quantitative parameter called the (true) ***equilbrium distribution coefficient*** or **q-value** to represent the equilibrium distribution ratio of a solute between two phases like the total cell water and the external bathing solution. In addition, I also introduced a **ρ-value** (Greek letter, rho, not English letter p) called the *apparent equilibrium distribution coefficient*. The q-value as a rule does not exceed unity (but there are exceptional cases of small excess beyond unity.) There is no limit on the magnitude of a ρ-value. However, if a ρ-value of a solute is substantially higher than unity, most of the solutes involved must be adsorbed—according to what is called the *surplus adsorption rule* (Ling 1992 p. 426.) Thus K^+ in living muscle cells may exhibit a ρ-value of 40. Since its q-value of K^+ is way below unity, all but 1% of the cell K^+ is adsorbed on β- and γ-carboxyl groups in frog voluntary muscle cells.

Since then, this area of research has really blossomed both in theory and in the variety and depth of worldwide experimental confirmation. As examples, Table 5.5 in my 1992 book (Ling 1992 p. 108–109) summarizes the work on the state of water in living cell and model systems in terms of: 1, solute distribution, 2, osmotic activity, 3, swelling and shrinkage, 4, freezing point depression, 5, vapor sorption at near saturation, 6, NMR rotational correlation time, τ_i, 7, Debye reorientation time, τ_D, 8, quasi-elastic neutron scattering. Without exception, all subjects studied have yielded support for the POM theory of cell water. Progress continued during and after 1992, including something extraordinary.

4.3.2 A new theoretical foundation for the polarized-oriented multilayer theory

Up to the turn of the century, none of the existing theories of multilayer polarization of water provided precise *quantitative* insight into how far the (effective) polarization can

reach. For this reason, I am very happy to have discovered a short cut and as a result introduced a new theoretical foundation for the POM theory in 2003 (Ling 2003.)

It was demonstrated theoretically that under ideal conditions, a checkerboard of alternatingly positively-charged P sites and negatively-charged N site at the precisely defined distance of 3.1 Å apart and called an ***Idealized NP Surface*** as illustrated in Figure 7, can polarize and orient multilayers of water molecules ***ad infinitum***. It should be recognized that electrical *polarization* or what Debye called *distortion polarization* plays a key role only at the first one (or perhaps an additional) layer(s) of water molecules; the *ad infinitum* long-range effect is due to self-propagating *orientation* or what Debye called *orientation polarization* (Debye 1929.)

Moreover, the theory also shows that water so (polarized) and oriented under ideal conditions, cannot be frozen at any *attainable* low temperature, which, as dictated by the Third Law of Thermodynamics, cannot go below absolute zero (Fowler and Guggenheim 1960 p. 224.) The prediction of non-freezable water was confirmed retroactively by work published half a century ago. Its authors, Canadian chemists, P.A.Giguère and K.B. Harvey (1956) were puzzled and could not explain the continued existence of water in its liquid state—as witnessed by its characteristic infrared absorption spectrum—in a 10 micra-thick layer of water held between polished silver chloride prisms at the temperature of liquid nitrogen and given by the authors as –176° C. I then discovered that AgCl crystals possess structures very close to that of the ***Idealized NP Surface*** (For AgCl crystalline structure given by Glaus and Calzaferri (1999), see Figure 9 in Ling 2003 on p. 118.) In

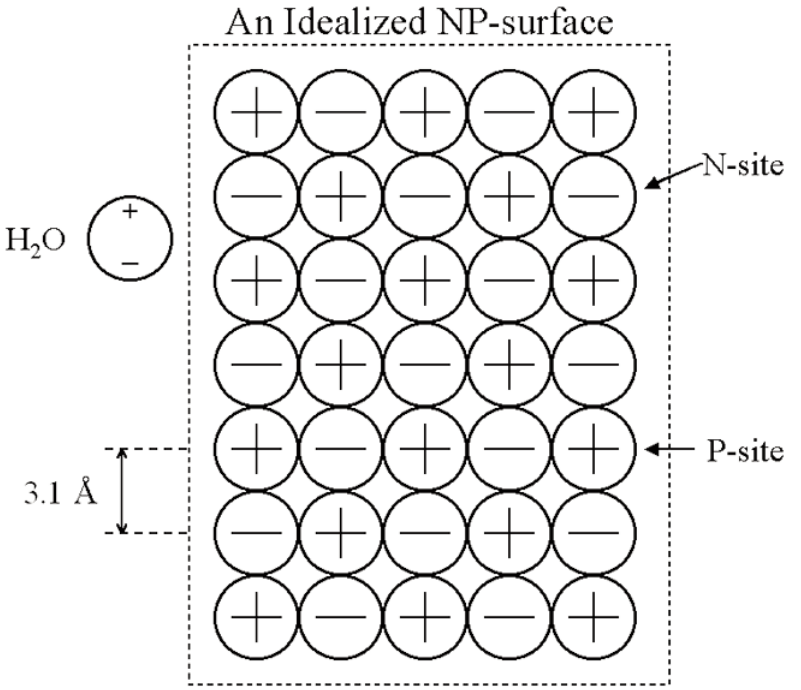

FIGURE 7. An Idealized NP surface. The distance between a pair of the nearest-neighboring N and P site is equal to the distance, r, between neighboring water molecules in the normal liquid state and equal to 3.1 Å.(From Ling 2003)

sharp contrast, no signs of non-freezable water were found in similar infrared spectrum of water held between (transparent) fluorite (CaF_2) plates with a different crystalline structure (Fox and Martin 1940.)

The prediction of long-range water (polarization) orientation was further dramatically affirmed experimentally by Zheng and Pollock in their publication on the "marching particles" near an NP surface of polyvinyl alcohol surface in the Phys. Rev. (Zheng and Pollock 2003.) To my surprise and disappointment, these authors did not refer to my paper on the *ad infinitum* water polarization (Ling 2003), nor the fact that a long list of linear polymers (including polyvinyl alcohol) are effective water dynamic structure inducers (Ling, Walton and Bersinger 1980.) (See also Pollack 2012.)

At the conclusion of this subsection, I would strongly recommend to the reader to download my 2003 article on "A New Theoretical Foundation for the Polarized-Oriented Multilayer Theory ..." by clicking Article No. 2 on the front page of my Website <www.gilbertling.org>. This article covers some of the most fascinating stories in the history of science.

4.3.3 The difference between freezing and vitrification of cell water

The dictum that under idealized condition, the dynamically polarized-oriented water cannot be frozen at any attainable temperature may appear in conflict with the phenomena of cryoprotection—where the addition of glycerol, ethylene glycol or other cryoprotectants keeps frozen living tissues alive at liquid nitrogen or helium temperature. Actually there is no conflict at all.

The word, frozen (living tissues) used here is not the freezing in the above-mentioned dictum, which means conversion of (normal or modified) liquid water into crystalline ice. The function of cryoprotectants is to prevent H_2O in the "frozen" tissue from turning into crystalline ice. So what is the physical state of water in the well-protected but solid living tissues in liquid nitrogen or liquid helium?

In 1937, Father (B. J.) Luyet first referred to this water as *vitrified* water. It was then thought to be amorphous and homogeneous. However, later work led to the idea of vitrified water as polymorphous. Based on the POM theory and the experimental findings of Ling and Zhang published in the early 1980's, I suggested that the vitreous state of living tissue cells kept at liquid nitrogen or liquid helium temperature is *vitrified polarized-oriented multilayer state* (Ling and Zhang 1983; Ling 1992 p.102–106; Ling 1992a pp.427–432; Zhang and Ling 1983.)

4.4 The association-induction hypothesis proper

The association–induction hypothesis is the one and only unifying theory of life at the cell and below-cell levels. It was published ten years after its embryonic prelude, Ling's Fixed Charge Hypothesis appeared in print in 1952. As pointed out earlier, for well over one half of a century, the association-induction (AI) hypothesis has successfully stood all the extensive testing here and abroad with no major setback. In fact, all criticisms of the theory known to me at the time (1998) have been answered (see Ling 1998d and Ling 1998e.) And with no known exception, what came as criticisms turned around and became additional supports for the theory (Ling 1998d and Ling 1998e.). I do not know of any additional criticism of the AI hypothesis published after 1998.

Notwithstanding, and as pointed out earlier, it is the alternative membrane theory or the membrane pump theory that is taught as truth worldwide at all levels of education—long after it has been soundly and unequivocally disproved (see p. 6 above.) Yet, among the advocates of the membrane (pump) theory could be counted some of the ablest scientists in history, including notably J. van't Hoff and A.V. Hill. In retrospect, I now see that they—like the *Reductionist Four*—also came on the scene before the ***microscopic*** approach in physics and chemistry became widely taught and practiced. As a result, they had no choice but to join the movement of interpreting life phenomena in terms of membranes, pumps, semi-permeability, channels, gates and other ***macroscopic*** concepts. It is no surprise that their respective talents notwithstanding, they failed.

Luckily, my generation of investigators arrived on the scene much later and thus in time to access the new science of *statistical mechanics*—invented primarily by Ludwig Boltzmann (Gurney 1949; Cohen 1997.) Thus privileged, I was able to construct a theory of life phenomena in terms of *microscopic entities* and named it the ***association-induction hypothesis***.

In the two preceding sections, we have gone to some details describing how some sites (β-, and γ-carboxyl groups) of the cell proteins interact with K^+ and other sites on the cell proteins (backbone NHCO groups) interact with the bulk-phase water. Based on the outline of protein chemistry and behavior, I shall describe how **the AI Hypothesis offers a self-consistent set of molecular mechanisms for the cell proteins to function as a coherent unit.** And that in turn enables the cell and its parts to stay alive and engage in life activities. We begin with the subject of the *target* groups and *effector* groups on cell proteins.

4.4.1 Target and effector groups

As in the case of chlorine atom-for-hydrogen atom substitution in acetic acid, a substitution of one atom or group of atoms linked to a molecule by ***covalent bonds*** produces the classic inductive effect. It is now well established that the substitution of one chemical group for another attached to the parent molecule by ***H-bond*** as well as by ***ionic bond*** also produces parallel inductive effects. The variety of the target groups for the inductive effect has also been broadened to include not only the pK_a of acidic groups, but also the pK_b of the basic groups, the strength of H-bonds and oxidation-reduction potentials (Ling 1984 pp. 183–198; Ling 1992 pp. 111–134.)

Figure 8 illustrates the influence of induction effect expressed by Taft's inductive constants of a list of effector groups on the pK_a of target carboxyl groups and pK_b of target amino groups close by and farther away. Figure 9 shows the inductive influence of similar effector group substitutions as expressed by Hammett's inductive constants σ on the strength of H-bonds as target groups. In both cases the effector groups are linked to the protein molecule by covalent bonds.

4.4.2 The transmission factor; the reach of Direct F-effect

The transmission of the combined inductive or I-effect (through intervening atoms) plus direct or D-effect (through intervening space) produces the F-effect. However, as time progressed, there have been more and more emphasis on the I-effect and less and less on the D-effect. Thus, in fact when I use the term Direct F-effect, it would be referring

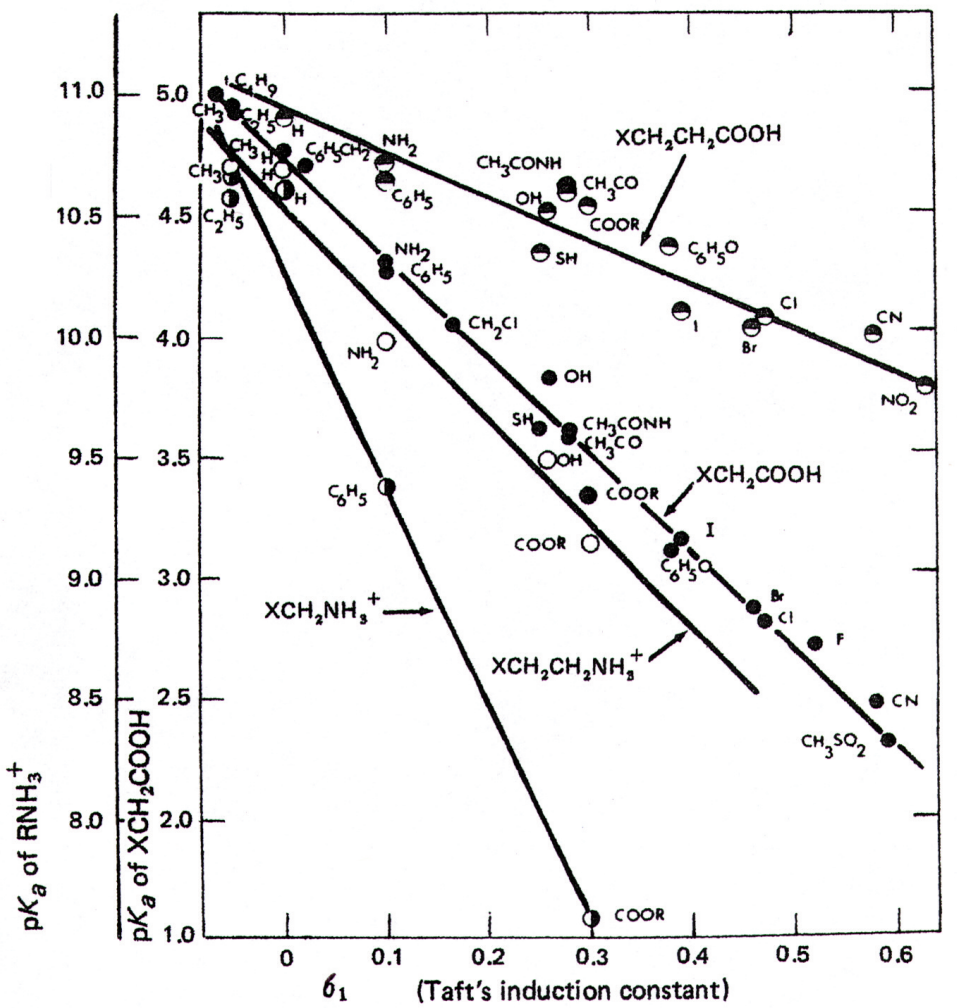

FIGURE 8. Linear relationships between the pK_a's of two sets of substituted carboxylic acids (acetic acid and n-propionic acid) and the pK_b's of two sets of substituted amines (methylamine and ethylamine) shown respectively on the double ordinates **and** Taft's induction constants, σ_1 of each of the substituents (marked as X in the structural formulae of the acids and amines) shown on the abscissa. (From Ling 1964)

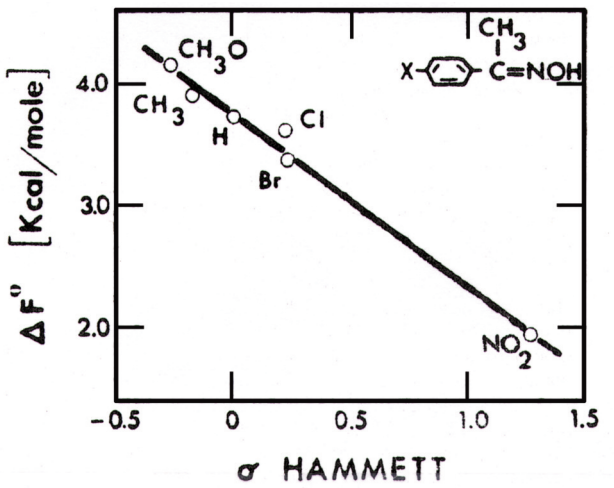

FIGURE 9. Relationship between Hammett's induction constants, σ's of the substitute X, on the free energy of dimerization of para-substituted acetophenone oximes. The structural formula of acetophenone oximes is shown in the upper right corner of the figure, where X represents the substituent. The free energy of dimerization shown as the ordinate was calculated from Reiser's data (Reiser 1959.) The fact that powerful electron donating substituents like the methyl group (CH_3) strongly enhances the strength of dimerization suggests that it is primarily the electron donating nitrogen atom (rather than the electron accepting OH group) that determines the strength of the H-bonds formed between a pair of the molecules (From Ling 1964)

largely if not exclusively to the I-effect mediated through intervening atoms. The ease or difficulty of the transmission of the inductive effect depends on what part of the protein molecule the transmission takes place.

The transmissivity for the passage of the I-effect through each saturated carbon atom is 0.333 according to Chiang and Tai (Ling 1984 p. 187.) However, others including Taft and Ling gave values as high as 0.48 (Ling 1964a; Ling 1984 p. 189.) Transmissivity through the peptide bonds is even more efficient by far.

Indeed, as shown in Figure 10, there are at least three sets of independent experimental data, which indicate that the Direct F-effect can be transmitted through three peptide linkages to reach a functional group directly or a functional group at the end of a short side chain. The details of the figure have been rewritten from those published earlier (Ling 2001 p. 161; Ling 1992 p. 125 and p. 132.) Why is the inductive or Direct F-effect transmitted so much more effectively through the polypeptide chain? The answer comes in subsection 4.4.3.1 below.

4.4.3 Transformation of proteins between two stable states

As pointed out repeatedly above, a unique feature of life is its connectedness. To achieve that end, I cited two models: the falling domino chain and the tethered frictionless seesaws. Both models can exist in two stable (or meta-stable) states only. In this subsection, I examine three different types of evidence to demonstrate the existence of physiologically active proteins in two stable discrete states also.

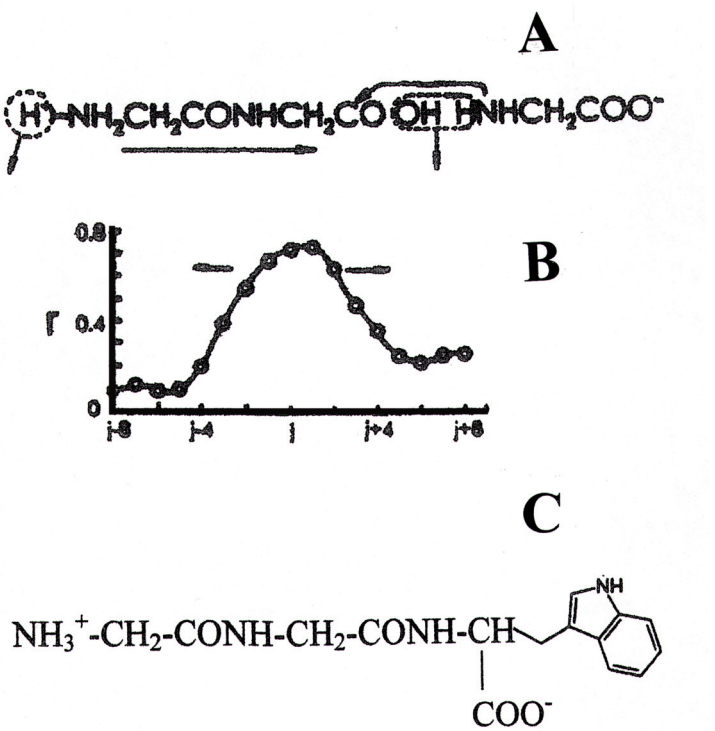

FIGURE 10. Three sets of experimental evidence for the effective transmission of the inductive effect through a length of the polypeptide chain.

(**A**) Demonstration of significant change of the affinity of the terminal amino groups of glycine and glycine peptides for H^+ as indicated by a significant decrease of its pK_a in response to a distant substitution of a carboxyl OH group by a stronger electron-withdrawing glycyl group at a minimum of ***three*** peptide linkages away (Stiasny and Scotti 1930; Czarnetzky and Schmitt 1931; Ling 1962 p. 94.)

(**B**) Linear correlation studies between the propensity of the peptide group of each of the 19 α-amino acid residues to form α-helical structure in proteins (i.e., *α-helical potential*) and the electron-donating power of their respective side chains (expressed as the acid dissociation constants of their α-carboxylic acid analogues) provided the data given in Table 1 on p. 46. Additional data given by Garnier *et al* made it possible to study the linear correlation (given as ordinate, r in Figure B) between the electron-donating strength of a specific amino acid residue referred to as the jth on the α-helical potential of the peptide linkage at different distance away from the jth residue. Thus as shown in the illustration, the positions labeled j-4 represents the peptide linkage of the 4th residue on the N-terminal side of the jth residue. On the other hand, the position labeled j+4 represents the peptide linkage of the 4th residue on the C-terminal side of the jth residue. The results as plotted in the figure show that the effect of each amino acid residue extends to at least the ***third*** peptide groups both upstream and downstream (Garnier *et al* 1978; Ling 1986; Ling 1992 p. 120.)

(**C**) Quenching of fluorescence of the indole group of the L-tryptophan residue (illustrated at the extreme right of the polypeptide formula shown) in a series of synthetic polypeptides of the general formula, $(glycine)_n$-L-tryptophan in consequence of the increasing distance created by the stepwise increase of the value of number of glycine residues, n separating the target indole group and the electron-withdrawing effector NH_3^+group, shown at the extreme left of the $(glycine)_2$-L-tryptophan formula. Best-fitting theoretical pH titration curves of the experimental titration data collected (not shown here) were based on the assumed value of pK_a for $(glycyl)_1$-L-tryptophan of 8.20, and the degree of fluorescence 50%, $(glycyl)_2$-L-tryptophan, 8.00, 21%, $(glycyl)_3$-L-tryptophan, 8.00, 10%. The data suggest that inductive effect can be effectively transmitted over ***three*** peptide linkages to reach the functional group on a short saturated carbon side chain. (Data from Edelhoch *et al* 1967; Ling 1992 pp. 124–125)

4.4.3.1 RESONANCE AND SHORT CN BOND OF THE PEPTIDE LINKAGE

N-methylacetamide, shown below in Equation 5, is the smallest molecule that contains a single *peptide linkage* (CONH.) As such, it is a useful model of one kind of building blocks of all polypeptide and proteins. In 1950 Mizushima and his coworkers demonstrated that the bond linking the N and C atom in this molecule is much shorter than a normal N-C single bond (Mizushima *et al* 1950; Mizushima *et al* 1955.) This bond-length shortening indicates ***resonance*** between two states as shown below:

$$\begin{array}{l} CH_2C\text{-}NHCH_3 \\ \quad\| \\ \quad O \end{array} \leftrightarrow \begin{array}{l} CH_3C{=}N^{+}HCH_3\,. \\ \quad\quad| \\ \quad\quad O^{-} \end{array} \qquad (5)$$

In consequence of this resonance, the CN bond in the peptide linkage is 40% double bond and 60% single bond. There is extremely rapid switching between the two structures of this most simple model.

The ease of transformation between two alternative structures makes the polypeptide chain of proteins highly polarizable electronically. It is this high polarizability that enables the polypeptide chain to serve as the "highway" of information and energy transfer over large distance like the frictionless seesaw chains (Ling 1962, p. 93.)

In addition, this resonance also makes the CO and NH groups function as *dipolar anion* and *dipolar cation* respectively. Dipolar ions are special because they are essentially neutral when seen at a far distance. However, at close range, they become either cationic or anionic, depending on which direction one is approaching the dipole. In the AI Hypothesis, these dipolar ions play critical roles in protein-protein interaction and in protein-water interaction.

4.4.3.2 INFRA-RED SPECTRA OF SYNTHETIC POLYPEPTIDES

In the early 1950's E.J. Ambrose and A. Elliott studied the infrared absorption spectra of synthetic polypeptides. And soon they made a very important discovery (Bamford *et al* 1956 p.130; Ambrose and Elliott 1951; Elliott 1953.) That is, in water, the polypeptide does not assume a large variety of conformations, as the popular term "random coils" would lead one to expect. Instead, each polypeptide assumes only one or the other of two alternative conformations. In one conformation, the NH and CO groups are oriented in the same direction as the polypeptide axis; this is exactly what one would expect if the conformation assumed is that of ***α-helical conformation***. In the alternative conformation, the NH and CO groups are oriented perpendicular to the polypeptide axis; this is exactly what one would expect if the polypeptide is in the ***fully-extended conformation***.

The results were so consistent and convincing that all the authors, but especially Elliott, went out of their (or his) way to make their conviction known. Notwithstanding, they could not see a greater significance beyond the observations until the association-induction hypothesis came along with what was first called the *biological fixed charge system* (Ling 1962 p. 53), then as the *minimal unit of life* (Ling 1992 p. 425)*, then* as the *elementary living machine* (Ling 2001 p. 152*)* and finally as *nano-protoplasm,*—which in theory also exists in two alternative conformations at equilibrium. In one, it is essentially an ***α-helical conformation*** and in the other it is essentially a ***fully extended conformation*** (Ling 2007.)

4.4.3.3 STRICT OBEDIENCE TO THE (TWO-STATE) YANG-LING COOPERATIVE ADSORPTION ISOTHERM

Boltzmann was a scientist of vital importance to the understanding of life and its physical basis because he almost single-handedly invented Statistical Mechanics. In addition, I also profited a great deal from another theoretical physicist of comparable rank, my dear friend, Professor C. N. Yang. He was my roommate at the Tsing Hua University Graduate School in Kun-ming, China between 1943 and 1945. (C.N. Yang and T.D. Lee were awarded the Nobel Prize for physics of 1957 for their conjoint work on parity. For a brief vignette of Yang's contribution to statistical mechanics, particle physics etc., see Yang 1995.)

Yang was among the first to read and endorse my 1962 book, "A Physical Theory of the Living State: the Association-Induction Hypothesis" long before it was published. Next thing you know, the Yang-Ling adsorption isotherm was born (Ling 1964a; Ling 1984 p. 208)—based on the one-dimensional Ising method. As such, it was a quantitative extension of my simple (two-state) model of a protein molecule seen as an infinitely long chain of equally spaced sites of similar nature, which could adsorb either an ith or a jth solute (where either the ith of the jth solute could be vacancy.) (For the details of the derivation of the Yang-Ling isotherm, see Karremann 1980.)

However, to fully understand how such a simple equation like the Yang-Ling adsorption isotherm can quantitatively describe the diverse aspects of living phenomena and their models requires space. Indeed, that was why the article "Nano-protoplasm, the Ultimate Unit of Life," cited as Ling 2007a is 123 pages long. To keep the present communication as short as possible, only one of those cases will be reviewed. For those interested in more, a click on Article #9 on the front page of my Website www.gilbertling.org and you have it in full.

Figure 11 demonstrates how the exceptionally accurate data of oxygen binding on hemoglobin by Dr. R.L.J. Lyster obtained in the laboratory of, and under the tutelage of Prof. F.J.W. Roughton of the Cambridge University of England can be precisely described by the Yang-Ling isotherm. All 16 data points fall on the theoretical curve dictated by just two numerical constants, an *intrinsic binding constant* of oxygen binding, $K^{oo}_{j\rightarrow I}$ equal to 5.88 x 10^{-6} M and a *nearest neighbor interaction energy* ($-\gamma/2$) equal to 0.67 kcal/mole (Ling 1969, but also reproduced in Ling 2007 Figure 17 on p. 150.) For a full discussion on the poignant significance of this precise prediction by two numbers, see Ling 2007a, pp. 147–152.

This high degree of quantitative agreement shown between theory and experimental data demonstrates that the protein can indeed exist in two stable states: in one, oxygen is bound in all the sites and in the other, the binding sites are all vacant.

The two-state model will be dealt with again in more detail below. But before that we will consider the chemicals that act like the little mouse shown in Figure 3, which can determine which of the two alternative conformations the living see-saw chain assumes. Drugs offer one category of such chemicals.

4.4.4 Cardinal adsorbents

In the association-induction hypothesis, drugs do not belong to a stand-alone group of chemicals. Rather, along with hormones, ATP, Ca^{++} etc., drugs are examples of ***cardinal adsorbents***, which exercise strong influence on living phenomena at low concentration. Cardinal adsorbents are electronic agents that play a central role in living phenomena.

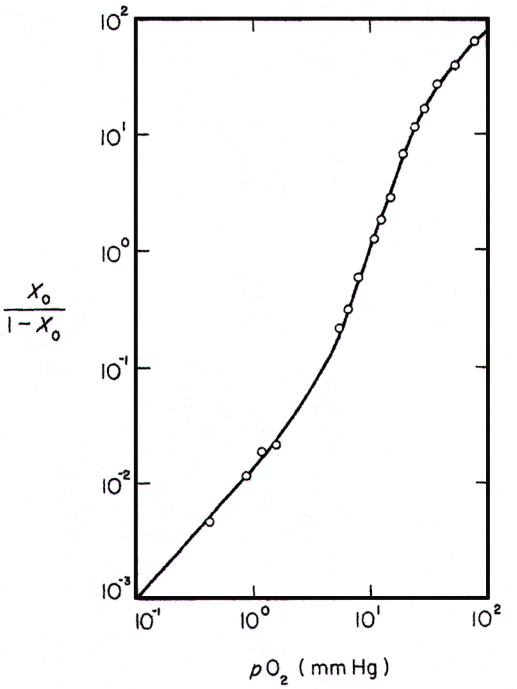

FIGURE 11. A log-log plot of the binding of oxygen by human hemoglobin in 0.6 M phosphate buffer at pH 9.1 and temperature of 19°C. Data from R. L. J. Lyster, as presented by Rossi-Fanelli *et al* (1964.) Points are experimental; line is theoretical according to the Yang-Ling adsorption isotherm presented as Equation 5 and 6 on page 142 in Ling (2007) with a $K^{oo}_{j\rightarrow I}$ equal to 5.88 x 10^{-6} M and (–γ/2) equal to 0.67 kcal/mole. (From Ling 1969)

Many drugs are made by Nature. Man makes others (Ling 1962 p.118 and p.420.) As a whole, cardinal adsorbents can be divided into three categories: *electron-withdrawing cardinal adsorbents* (**EWC**), *electron-donating cardinal adsorbents* (**EDC**) and *electron-indifferent cardinal adsorbents* (**EIC**).

A cardinal adsorbent as a rule has the *three-in-one power* to bring about (i) ***from here to there,*** (ii) ***one on many*** and (iii) ***making many respond as*** if they were one—when it binds onto a specific site on a cell protein called a ***cardinal site***. Cardinal sites include what we conventionally call *receptor sites* (for external molecular agents like drugs, hormones) but they also include other sites that interact with internal molecular agents like ATP (see below.) To demonstrate the three-in-one power of key cardinal adsorbents, I shall talk about three special cardinal adsorbents: Adenosine triphosphate or ATP is what I sometimes call the Queen of cardinal adsorbents for its overreaching power as an EWC. Ca^{++} is another important EWC. Ouabain is yet another powerful cardinal adsorbent but an EDC.

4.4.4.1 ATP

Discovered by K. Lohmann in 1929, ATP was for about 15 years widely believed to carry two high-energy phosphate bonds, each represented by the symbol ~P in a theory

proposed by Lipmann (Lipmann 1941.) This concept turned out to be mistaken as shown by Podolsky and Morales (1956.) They found no usable energy to do work in any one of the three phosphate bonds (Ling 1992 p. 179; Ling 2001 p. 234, 306.) ***This iconoclastic development left ATP without a function—***for six years only or ***altogether***, depending on one's familiarity with or lack of it with the association-induction hypothesis.

That apparently all the textbooks at high school and university level worldwide has ignored Podolsky and Morales's historical discovery shows that the inability to deal with major progress is not limited to my work but is an illness affecting the whole science. As such, it provided the legitimate causes for sentiments expressed in Chalmers's book, *What Is This Thing Called Science?* And other negative sentiments toward the future of science shared among some forty leading scientists in different fields, including science philosophers, Sir Karl Popper, Thomas Kuhn and Paul Feyerabend in John Horgan's book, *The End of Science: Facing the Limits of Knowledge in the Twilight of the Scientific Age* (Horgan 1996.) However, in the Preface of my 2001 book, *Life at the Cell and Below-Cell Level*, I pointed out that science has not come to an end, the mistaken notion was created in part by the failure to recognize the association-induction hypothesis, which gives ATP a new function as an important cardinal adsorbent. As such, it is distinguished by its strong binding energy with a standard free energy of binding, ΔF° equal to –14.3 Kcal/mole (Ling 1992 p. 180.) Thus the binding energy on myosin of ATP is ten times higher than the binding energy on myosin of its hydrolytic product, ADP (Ling 1992 p. 187.)

As the Queen of electron-withdrawing cardinal adsorbent (EWC), ATP has been demonstrated to show a stoichiometric relationship to the level of K^+ in a variety of living cells studied (Ling 1962 pp. 252–255) but most extensively in frog muscle cells. In this case, the same quantitative equilibrium relationship of 20 K^+ selectively adsorbed for each ATP occupying its specific cardinal sites persists regardless of which one of the eleven different poisons is employed to bring about the decline of ATP level very slowly. They include iodoacetate, 2,4-dinitrophenol, azide and chlorpromazine (Gulati, Ochsenfeld and Ling 1971; Ling 1992 p.189; Ling 2001 p. 72.)

ATP also shows stoichiometric relationship to intracellular Na^+ (and sucrose) concentration level. (For supporting experimental evidence, see Figure 8.18 and Figure 8.19 in Ling 1992; Figure 56 in Ling 2001.)

Since all sucrose in the muscle cell and (virtually) all intracellular Na^+ are dissolved in cell water, the control of ATP on their concentrations is achieved via its control of the dynamic structure of bulk-phase cell water. Now each kilogram of normal resting frog muscle contains 80% by weight or 44.4 moles of water and 5 nmoles of ATP. Resting frog muscle cell water has a q-value of 0.132 for sucrose and 0.100 for D-raffinose (Ling, Niu and Ochsenfeld 1993, p. 191.). A simple calculation shows that at least 40 moles of water in one kilogram of muscle cells are under the control of ATP. Put differently, each molecule of ATP adsorbed on its cardinal site controls at least eight thousand (8000) water molecules.

4.4.4.2 Ca^{++}

Like ATP, Ca^{++} is what I call a "*conservative cardinal adsorbent*" because its function is to maintain the resting physiological state of the cell rather than pushing the cell to a different active state (Ling 1992 pp. 171–172.) In agreement with this view, lowering external Ca^{++} concentration experimentally causes massive loss of cell K^+ in brain slices (Gardos 1960), liver slices and transplanted tumors (Gilbert 1972), carotid arteries (Jones

1973) and guinea pig *taenia coli*, a strip of smooth muscle along side of the intestine (Gulati 1973.) No further study of the role of Ca^{++} in terms of the AI Hypothesis has been reported since the early 1970s to the best of my knowledge despite the obvious success in work done and reported earlier.

Meanwhile, the textbooks of biology worldwide continue to teach that living cells are sacs of free watery solutions enclosed in cell membranes containing an unlimited number of inward and outward pumps. Among these membrane pumps studied extensively is the mighty Ca pump. It is allegedly able to maintain in human red blood cells an extracellular/intracellular concentration gradient as high as 45,000. (Bogdanova *et al* 2013.) Is this real?

Keeping in mind that none of these (membrane) pump concepts can be even called a scientific hypothesis—because by definition a scientific hypothesis provides a mechanism for the phenomenon observed. A much more extensively studied hypothetical membrane pump is the sodium pump. Glynn and Karlish (1975) who wrote the first of its kind of review under the title "The Sodium Pump" readily admitted that no mechanism for the pump has ever been proposed. Thus, even the so-called Na^+ pump hypothesis is only a rephrasing of an observation.

True, pumping of ions and molecules does occur in living systems but only in *bifacial* cells like kidney epithelium and frog skin (Ling 1981a.) For *unifacial* cells like frog muscle and human red blood cells, none survived careful testing. Indeed, what the proponents of the Ca pump have been doing more recently are almost exact duplicates of what have been done in the early days on the sodium pump.

As mentioned earlier on p. 6, I demonstrated in 1962 that the postulated sodium pump would require at least 15 to 30 times of the total energy the muscle cells command under the condition of the experiment. But that estimate was made before the demonstration of Morales and Podolsky that ATP does not carry extra usable energy in the so-called high energy phosphate bonds. When this fact is taken into account, the discrepancy between the maximum energy available and minimum energy needed would be far beyond a mere 15 to 30 times. Or put it in a simple way, the maintenance of a low Na^+ level in (muscle) cells simply does not require a continual supply of any amount of energy. The fact that we can preserve all kinds of living cells, including the human red blood cells and animal embryos containing all kinds of tissue cells, in liquid nitrogen or liquid helium indefinitely, leaves no room for arguments.

In 1980, Ling and Negendank also asked the question that investigators of the Ca pump have been asking years later: Do isolated vesicles pump sodium and the answer was no (Ling and Negendank 1980.) Does man-made phospholipid membrane-containing the alleged pump (Na, K-activated ATPase) pump sodium and potassium? The answer is again no (Ling 1992 pp. 22–24.) Does K^+-selective microelectrode faithfully tell us about the free K^+ concentration in living cells? The answer is again no (Ling 1984 pp. 252–257.)

But even these are not all. Other studies have shown that any intracellular ion-specific electrode can only see the concentration of a thin layer of water coming from the damaged protoplasm that the intruding electrode has produced. Thus in some way, the activity of ions and molecules inside living cells are like the life or death of the cat in Maxwell's black box. Opening the door to find out if the cat is alive will inevitably trigger the release of poison that instantly kills the cat and defeats the purpose of finding its state of health before opening the door. Inserting an ion-selective electrode or injecting a

dye, a photoprotein (e.g., aequorin), a metallochromic indictor (e.g., fura or quin 2) will as a rule produce unpredictable changes in the free Ca^{++} concentration and its state of binding from their respective natural state. Notwithstanding, there are indirect and proven and published ways of accurately determining the concentration of free and adsorbed ions or neutral molecules inside living cells but there is no evidence that investigators of the Ca^{++} pump are aware of their existence and take advantage of them.

4.4.4.3 OUABAIN

As a specific example of the far-reaching power of another cardinal adsorbent, this time an EDC, consider an experiment that Ling and Bohr conducted in 1971 (Ling and Bohr 1971.) We sterilely isolated frog sartorius muscles and incubated them at 25°C in a gently shaken known volume of sterile modified Ringer solution, which contained 2.5 mM K^+ and 100 mM Na^+ (Ling and Bohr 1969.) We then added to the solution in each one of half of the flasks a minute amount of the drug, ouabain to reach a final concentration of 3.26 x 10^{-7} M. After 72 hours of sterile incubation at 25°C, we took out the muscles and analyzed their ionic contents. Whereas the normal control muscles retained all their K^+, the muscles exposed to ouabain had quantitatively replaced one-on-one all their K^+ with Na^+. These findings provided us with the exact information on how many β-, and γ-carboxyl groups in the muscle protein(s) have changed the kind of its adsorbed cations. Assuming all the ouabain added to the incubation fluid to be adsorbed on its appropriate cardinal sites, we calculated that the binding of ***a single ouabain molecule*** has made ***one thousand and forty-two*** (1042) β-, and γ-carboxyl groups in the muscle protein(s) to shift from adsorbing K^+ to adsorbing Na^+ (Ling 2001 p. 262.) How such a far-reaching switch can be accomplished in molecular and electronic terms will be the conceptual cement joining the subject matters of the three subsections following.

4.4.5 c-value, c-value analogue etc.

As described in an earlier page, the pK_a is a convenient parameter representing the affinity of an acid for its H^+. Weak acetic acid has a pK_a of 4.76 while strong TCA has a pK_a of only 0.66. In presenting Ling's Fixed Charge Hypothesis, I have shown that the mechanism suggested for the selective accumulation of K^+ over Na^+ in living cells can be extended to explain selective uptake of K^+ in non-living systems as well. They include soil, glass as well as man-made *ion exchange resins*, which too selectively accumulate K^+ over Na^+ —although at selectivity ratios far below that seen in living cells (Ling 1952; see also Wiegner & Jenny 1927; Jenny 1932; Ling 1962 p. 56.) A later type of ion exchange resin, however, selectively accumulates Na^+ over K^+. In Bregman's review on the subject, he pointed out that the earlier form of ion exchange resin that prefers K^+ carries anionic sulfonate groups while the later type of resin that prefers Na^+ have anionic carboxyl groups (Bregman 1953.) Of course, the sulfonate group is a much stronger acidic group than the carboxyl group. Bregman, however, did not make anything out of this insight, preferring a difference in the *polarizability* of the different acidic groups as suggested in the theory of Teunissen and Bungenberg de Jong (1939.) However, I became very excited when I learned of this selectivity reversal in the new type of ion exchange resin.

There was a special reason for my interest in this selectivity reversal. I had been invited to join the basic research staff of the newly founded Eastern Pennsylvania Psychiatric

Institute in Philadelphia. And before my acceptance of the job, I had given a talk to my prospective colleagues, Donald Rudin, George Eisenman and James Casby. Included in the talk was my theory of selective accumulation of K^+ in living cells (Ling 1952) as well as the role of selective adsorption in generating the electric potential difference across the surface of nerve and muscle cells (Ling 1955.)

Some time afterward, Eisenman, Rudin and Casby suggested a new modification of my earlier theoretical model. They thought that if the field strength of the fixed negative charge does not stay put as in my original model but changes, then among the five alkali-metal ions, cesium (Cs^+), rubidium (Rb^+), potassium (K^+), sodium (Na^+) and lithium (Li^+), the most weakly hydrated Cs^+ will be the first to lose its hydration, followed by Rb^+ etc. so that 11 orders of the five alkali metal ions will be created (Eisenman *et al* 1957; Eisenman 1967; Ling 1984 p.153.) Extremely important as it was to me at the time, their theory was nonetheless entirely speculative. For that reason, I felt that the best way to proceed from there on was to do a detailed quantitative study on the subject. And to begin, I needed to invent a new independent parameter that underlies the pK_a value. The result is what I call the **c-value.**

Rigorously defined in my first book, *A Physical Theory of the Living State*, the c-value represents the electron density of the *negatively-charged oxygen atom* of an oxyacid like a β-, or γ-carboxyl group but given in the distance parameter of Angstrom units (Ling 1962 pp. 57–60; Ling 1984 pp. 155–156; Ling 1992 pp. 126–127.) A high c-value corresponds to a high electron density and a high pK_a ; a low c-value corresponds to a low electron density and a low pK_a. In contrast, the carbonyl oxygen atom of a peptide NHCO group is the *negative end of an electric dipol*e. A different parameter is introduced for its effective strength called the **c-value analogue** (Ling 1962, p. 57, 60.) Two other parameters were also introduced. The **c'-value** refers to the positive charge of a positively charged amino or guanidyl group and the **c'-value analogue** of the positive charge of dipolar peptide imino group, for example.

4.4.5.1 THEORETICAL PREDICTED REVERSAL OF K^+ / NA^+ PREFERENCE WITH C-VALUE CHANGE

With the c-value defined, I then decided to construct a **linear model**, in which a cylindrical cavity is carved out of the continuous dielectric of bulk-phase water. An array of interacting ions and water was then installed in the cavity. A singly charged oxygen atom is placed on one end of the cavity and one of the seven mono-valent cations: Li^+, Na^+, K^+, Rb^+, Cs^+, H^+ and NH_4^+ is placed on the other end of the cavity, separated from the oxygen atom by 0, 1, 2 or 3 water molecules. There are also two additional water molecules beyond the mono-valent cation. Instead of the simple Coulombic interaction considered in my 1952 model, seven other types of interactions were taken into account (for details see, Ling 1962 pp. 60–74.) The polarizability (α) of the oxyacid group was given three values (0.876×10^{-24}, 1.25×10^{-24} and 2.0×10^{-24} cm^3.) Only the results from the model with the highest oxygen atom polarizability (α) is shown in Figure 12 here.

Broadly speaking, the theoretical result confirms the idea of Eisenman, Rudin and Casby that with the increase of field strength, (given here as the c-value) there is a sequential change in the preference for any pair of two mono-valent cations considered. In contrast, by ignoring the polarizability or α value, Eisenman's simple model cannot account for the high H^+ over K^+ preference observed in virtually all kinds of living cells examined. Yet, this is accurately predicted with the model shown in Figure 12 with an

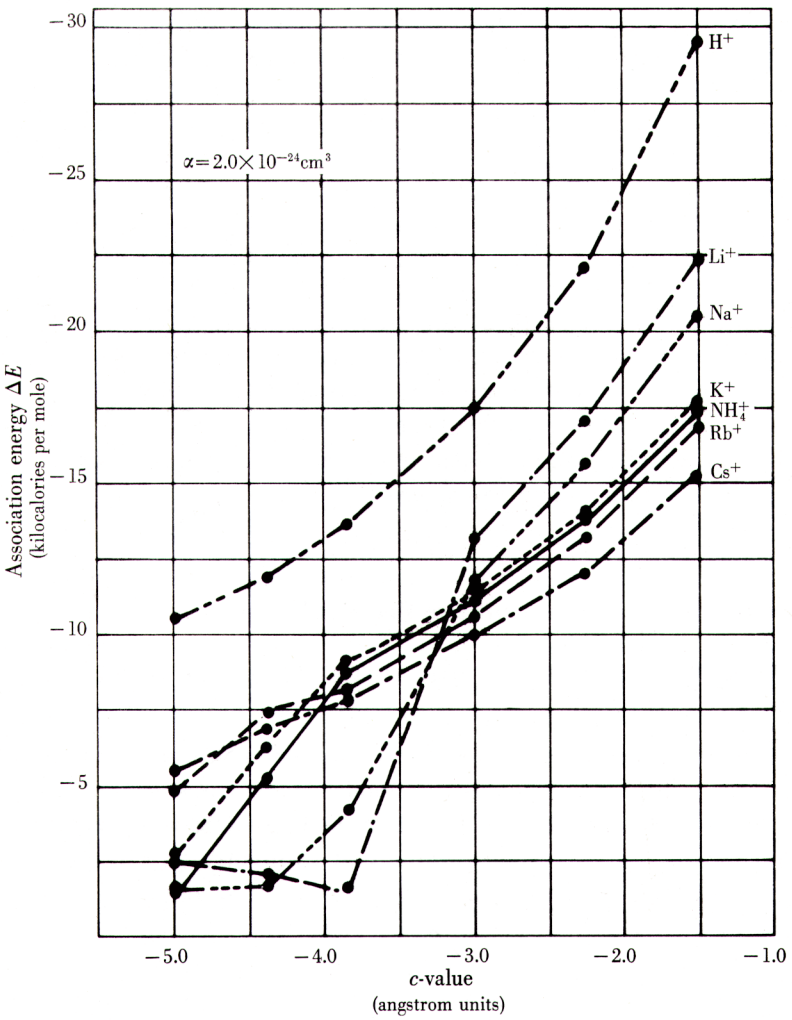

FIGURE 12. The theoretically computed association-energies in kilogram calories per mole are shown on the *ordinate* for six monovalent cations on a singly-charged oxyacid group with a polarizability of 2.0 x 10^{-24} cm^3 and c-value as indicated on the *abscissa*. An ion, say K^+, which shows a higher negative energy of association of –8.3 kcal/mole on a fixed oxyacid at the c-value of –4.0 Å is preferentially adsorbed over Na^+, which at the same c-value shows a lower negative association energy of –3.3 kcal/mole only. However, at a higher c-value of –2.5 Å, the preference is reversed since at this point the negative association energy of Na^+ at –16 kcal/mole is higher than that of K^+ at –13 kcal/mole. Preference reversal at different c-values is important in physiological activities according to the association-induction hypothesis as described in Figures 13 and 14 following. (From Ling 1962)

α value of 2.0 x 10^{-24} cm^3. However, the results from the two additional ions H^+ and NH_4^+—also not dealt with in Eisenman, Rudin and Casby's model at all—are of particular significance. Since all the fixed cations carried on proteins are variants of the NH_4^+ ion, the computed values for this ion as shown in Figure 12 offers more than its face value. That is, it also offers insight in the way c-value changes can alter the salt-linkage formation between fixed anions (mostly β-, and γ-carboxyl groups) and fixed cations (a-amino groups, ε-amino groups and guanidyl groups) in protoplasm.

4.4.5.2 THEORETICAL PREDICTION OF SWITCHING BETWEEN α-HELICAL ↔ FULLY-EXTENDED CONFORMATION WITH CHANGE OF C-VALUE ANALOGUE OF PEPTIDE CARBONYL OXYGEN ATOM

C.B. Anfinson shared the 1972 Nobel Prize for Chemistry for the discovery that the protein *ribonuclease* denatured in a concentrated urea solution, can be completely returned to the original so-called "native state" by washing away all the urea taken up by the protein (Anfinson 1967.) (For evidence that this assignment of "native" state is inverted and thus erroneous, see Ling 2001, Sect. 16.6 (1.3) on pp. 243–246 and p. 314; Ling 2006, Sect. 2.5 on pp. 10–15.) From this observation, Anfinson concluded that the folding patterns, or secondary and higher structures are determined by the protein's unique amino acid sequence also called the *primary structure*. But he made no suggestion how the primary structure determines the secondary and tertiary structure. The AI Hypothesis as described in pages following fills this conceptual gap and in the process has corrected a major error in protein chemistry on what is truly native and what is denatured. But as far as I can tell, rank-file protein chemists have not paid any attention to the suggested changes yet.

In the preceding section, we have shown how the effective electron density as expressed by the c-value determines the strength of a specific mono-valent cation's adsorption. As also mentioned above, all the fixed cations of a protein—be it an α-amino group at the N-terminal of a protein chain, or an ε-amino group carried on the side chain of a lysine residue or a guanidyl group at the end of an arginine residue—are all modified ammonium ion (NH_4^+.) Since what are known as salt linkages are formed between pairs of fixed cations and fixed anions, and in the AI Hypothesis salt linkages constitute the dominant component of the tertiary structure of a protein, clearly the c-value of the fixed anions determines the tertiary structure in the AI Hypothesis. By analogy, I suggested in the AI Hypothesis that the effective electron density of the peptide carbonyl oxygen atom determines the secondary structure as a mix of α-helical structure and fully extended structure (Ling 1986.) Table 1 shows how satisfactorily confirmed this idea turns out to be in the end. It has been retroactively confirmed by the quantitative data from three independent groups of prominent investigators: Chou and Fasman (1978); Tanaka and Scheraga (1976); Garnier, Osgathorpe and Robson (1978.)

Now the propensity or potential of the NHCO linkage of a specific amino acid residue to form an α-helical structure is called *α-helical potential.* The data shown in Table 1 yield respectively the positive linear correlation coefficients of +0.77 (Chou and Fasman 1978), +0.75 (Tanaka and Scheraga 1976) and +0.72 (Garnier *et al* 1978), averaging +0.75 between the α-helical potentials of 19 amino acid residues and the *electron donating strength* of the amino-acid-residues's side chains—as revealed by the pK_a of the corresponding carboxylic acids (e.g., formic acid for glycine; acetic acid for alanine.) The

TABLE 1. The α-helical potentials from Chou and Fasman (1978), Tanaka and Scheraga (1976) and Garnier, Osguthorpe and Robson (1978) of 19 α-amino acids and the pK_a of the corresponding 19 carboxylic acids. The linear correlation coefficients of Chou and Fasman's series of α-helical potentials and the pK_a's is +0.77. That derived from the Tanaka and Scheraga series and the Garnier *et al* series are +0.75 and +0.72 respectively. The average of all three sets of linear correlation coefficients is +0.75. (From Ling 1986)

	Chou and Fasman (P_α)	Tanaka and Sheraga ($\omega_{h,j*}$)	Garnier et al. (j)	Corrected pKa of Analogous Carboxylic Acids
Glu (-)	1.51	1.188	164	5.19
Ala	1.42	1.549	151	4.75
Leu	1.21	1.343	118	4.77
His (+)	1.00	0.535	98	3.63
Met	1.45	1.000	139	4.50
Gln	1.11	0.795	96	4.60
Trp	1.08	1.105	98	4.75
Val	1.06	1.028	100	4.82
Phe	1.13	0.727	102	4.25
Lys (+)	1.16	0.726	109	4.70
Ile	1.08	0.891	92	4.84
Asp (-)	1.01	0.481	91	4.56
Thr	0.83	0.488	60	3.86
Ser	0.77	0.336	47	3.80
Arg (+)	0.98	0.468	77	4.58
Cys	0.70	0.444	73	3.67
Asn	0.67	0.304	35	3.64
Tyr	0.69	0.262	41	4.28
Gly	0.57	0.226	0	3.75

rule is that the higher the pK_a of the corresponding carboxylic acid, the greater is the propensity of the CONH group of that amino acid residue to engage in α-helical structure. Or in the lingo of the AI Hypothesis: *the higher the c-value analogue of the peptide carbonyl oxygen atom, the greater is the probability of that amino acid residue's peptide group forming α-helical structure.*

Our next question is an equally fascinating one. What is the alternative of a peptide linkage if it does not form an α-helical structure? Once more, the AI Hypothesis offers an answer decisively different from the conventional one.

The conventional answer is that it will become a part of a random coil. I believe that this is highly questionable. For example, the infrared absorption studies of Ambrose and Elliott (1951), especially of Elliot, mentioned above show that in an aqueous medium, the polypeptide exists only in one or the other of just two alternative conformations: the *α-helical conformation* and the *fully-extended conformation,* a conclusion I cited from these authors in Section 4.4.3.2.

Since we have already established that a protein in the fully extended conformation adsorbs and polarizes multilayers of water molecules, clearly a protein's two alternative choices are: (1) existing in the fully extended conformation and adsorb deep layers of water molecules or (2) assuming the *α-helical conformation* mixed with liberated free water molecules. And the data given in Table 1 demonstrate *that high c-value analogue favors the α-helical conformation whereas low c-value analogue favors the fully extended conformation—with multilayer adsorption of water molecules through self-propagating (polarization and) orientation.*

4.4.6 The AI cascade mechanism (until the year 2007, known as Indirect F-effect)

As pointed out earlier, a pervasive trait of living matter is its connectedness. We know that the transmissivity through a saturated carbon atom is 0.33 or as high as 0.48 and that the static Direct F-effect can transmit through three peptide linkages directly or in addition to a short saturated carbon chain. However, to achieve what underlies its triple (i) ***one on many,*** (ii) ***from here to there*** and (iii) ***making many respond as one*** capabilities, the AI Hypothesis offers what was once called the *Indirect F-effect* until the year 2007. From 2007 on, it has been given the new name, *AI Cascade mechanism* (Ling 2007a.) As in the title of the AI hypothesis, A and I stand for association and induction respectively. And you will soon find out why.

Once more I repeat an earlier declaration to present here an essentially unmodified narrative on the model of long-range information and energy transfer that I presented first in my 2001 book (Ling 2001 pp. 147–149; Ling 2007.)

The single inset in Figure 13 illustrates the type of relationships between c-value analogue and c'-value analogue and the alternative adsorbents. At higher c-, or c'-value analogue, a^+ and a^- are respectively preferred. At lower c- or c'-value analogue, b^+ and b^- are respectively preferred. Now, let us suppose that as shown in Figure 13-A, initially all the backbone CO groups shown as O and all the backbone NH groups shown as H are respectively at the high c-value analogue and c'-value analogue of 2. In Figure 13-B and 13-C, the adsorption of the EWC, **W** at the cardinal sites withdraws electrons from the nearest neighboring O site, decreasing its c-value analogue from 2 to 1. This decrease of the c-value analogue reverses the preference for a^+ over b^+ to b^+ over a^+. As a result, the a^+ originally occupying the O site is replaced by a b^+.

Now b^+ is a weaker electron-withdrawing agent than a^+. In consequence, the displacement of a^+ by b^+ releases electrons. Some of the released electrons go back upstream toward the cardinal site-**W** couple, enhancing its electron-withdrawing effect. Other electrons released go downstream to the nearest H site, causing a decrease of its positive charge and hence a fall of its c'-value analogue from 2 to 1. A reversal of its preference for a^- over b^- to b^- over a^- follows, leading to the displacement of a^- by b^-. Now b^- is a weaker electron donator than a^-. In consequence, the displacement of b^- for a^- withdraws electrons. Some of the electrons withdrawn come from the O site upstream, further decreasing the c-value analogue of the O site upstream. Some of the electrons come from the next O site downstream, lowering its c-value analogue from 2 to 1. A b^+ for a^+ exchange follows. And the cycle repeats itself until all the a^+ and a^- are replaced by b^+ and b^- respectively as shown in Figure 13 C.

In Figure 13 the side chains are not represented. In Figure 14 we take into account functional groups on short side chains also.

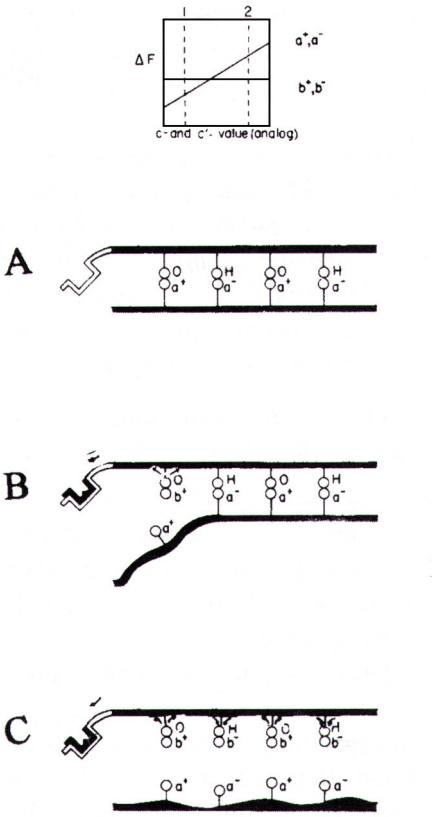

FIGURE 13. A theoretical model showing how adsorption of a cardinal adsorbent **W** on a controlling cardinal site of a protein brings about an (controlled) auto-cooperative transition resulting in an across-the-board uniform change in the electron density of the backbone NH and CO groups.
A sterically and electronically specialized region at the left end of the upper protein chain shown as a dark horizontal line makes up a *cardinal site*. Empty circles attached to the protein chains and labeled H and O represent respectively backbone NH and CO groups. Inset diagrammatically illustrates how changes in the *c-value analogue* of backbone CO groups alters the relative affinities for adsorbent a^+ and b^+ and how changes in the *c'-value analogue* of backbone NH groups alter the affinities for adsorbents a^- and b^-. See text for a description of how adsorption of the electron-withdrawing cardinal adsorbent **W**, on the cardinal site creates an inductively propagated across-the-board and uniform change in all the backbone NH and CO groups. (From Ling 2001)

In Figure 13 we treated adsorption on the backbone O and H individually. In Figure 14, however, each pair of adsorbent a^+ and a^- in Figure 13, (adsorbed respectively on the CO and NH groups of peptide groups belonging to a single amino acid residue) is treated as a single entity and represented by a rectangular box a. Figure 14A then represents a protein segment before an EWC is taken up. Here the electron-withdrawing power of the backbone cationic component (a^+) is represented as a downward arrow in the rectangular box, while the electron-donating power of the anionic component (a^-) is represented by an upward arrow.

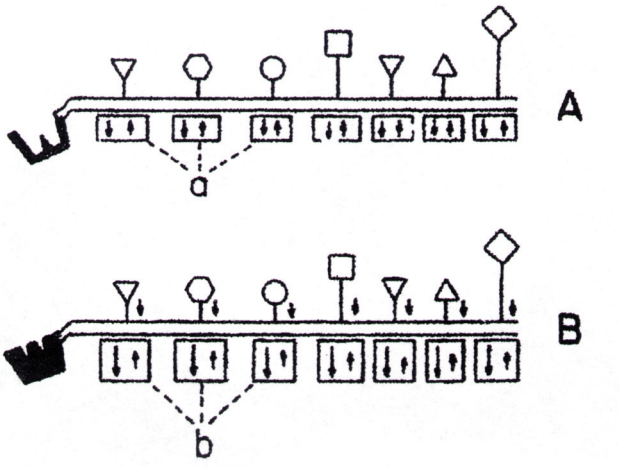

FIGURE 14. An alternative view of seeing the model of controlled auto-cooperative transition presented in Figure 13 above with focus on the impact of the electron-withdrawing cardinal adsorbent (EWC) shown as **W** on the various functional groups on side chains (shown as triangles, hexagons, circles and squares.) Displacement of the weaker (net-) electron-withdrawing **a** (representing Figure 13's a^+ and a^- together as a single net-electron-donating or a single net-electron withdrawing unit) on each peptide NHCO group shown in **A** by the stronger net electron-withdrawing **b** (representing Figure 13's b^+ and b^- together here as a single electron-donating or electron-withdrawing unit) shown in **B** is initiated by the adsorption on the cardinal site of the electron-withdrawing cardinal adsorbent (**W**.) This adsorption of **W** leads eventually to the across-the-board decrease of electron density (shown by downward arrows) of all side chain functional groups. Length of the upward arrow in the rectangular boxes indicates strength of the *electron-donating* effect; length of the downward arrow in the rectangular boxes (and along the side chains) indicates the strength of *electron-withdrawing* effect. For more details, see text.

Figure 14B shows the same protein segment after an EWC, **W,** occupies the cardinal site. As a result, the sequence of events described under Figure 13A to C takes place. And box b (which stands for b^+ and b^- together) now displaces box a (which stands for a^+ and a^- together). Since the O site, shown in Figure 13, to represent a carbonyl group (CO) is highly polarizable but the H site shown in Figure 13 to stand for an imino group (NH) is much less polarizable (Cannon 1955; Mizushima *et al* 1955), the *electron withdrawing effect* at the O site is strong and represented by a long downward arrow in the b box. In contrast, the *electron-donating effect* at the H site is weak and is represented as a short upward arrow. The *net effect* of displacing box a by box b is therefore an electron-withdrawing effect. Since this electron-withdrawing effect is repeated at each CONH peptide group, every functional group experiences a similar electron withdrawing influence as indicated by the downward arrows of the same length and in the same direction along each side chain as shown in Figure 14B on top of the polypeptide chain shown as a double narrowly-separated straight line. Of course, the functional groups at the end of the side chains differ and are diagrammatically represented variously as triangles, squares and circles. The functional groups that are on short side chains undergo the greatest change.

Now most abundant functional groups on short side chains are β-, and γ-carboxyl groups. A lowering of their electron density means a fall of their c-value (from an initial

high value.) As a result, a monovalent cation A^+ may be replaced by a B^+. This B^+ for A^+ exchange in turn reinforces the b for a exchange at the backbone as well as the adsorption of the EWC at the cardinal site. *Taken together, the backbone sites and the functional groups on short side chains and their respective adsorption partners (a/A^+/etc. versus b/B^+/etc.) constitute the alternative basic components of the auto-cooperative assembly diagrammatically illustrated in Figure 14B.*

In consequence of the interaction with the EWC, **W**, the backbone CO groups as well as the β-, and γ-carboxyl groups (and other functional groups on short side chains) undergo an across-the-board ***electron-density decrease*** from their initial higher values. And an exchange of adsorption partners occurs at both sets of sites. Conversely, with an EDC adsorbed onto the cardinal site of a protein segment, the backbone CO groups as well as the β-, and γ-carboxyl groups (and other functional groups on short side chains) may undergo an across-the-board uniform ***electron density increase*** from their initial lower values. And exchange of adsorption partners follow at both sets of sites in the reverse direction.

On account of their coherence, one may say that the whole "gang" of cooperatively linked functional groups on short side chains and the backbone carbonyl groups behave as if it were a single site. By the same token*, three-in-one impacts* of (i) *one-on-many*, (ii*) from here to there* and (iii) *making many sites behave as one site* capabilities produced by the binding or release of a cardinal adsorbent have all been achieved.

5 What is life

Charles Dickens wrote *Great Expectation* to tell about life in Victorian England. Cao Xue Qin wrote *Red Chamber Dreams* to tell about life of an aristocratic family in 18th century China. Leo Tolstoy wrote *War and Peace* to tell about life in Russia during the Napoleon invasion. In all these stories what I call life refers to social life. To portray social life, the writers tell about the lives of individuals, be it Pip in one, Jia BaoYu in another and Pierre in still another. The guiding rule is to explain life of a larger living entity in terms of the life of an entity one level smaller.

Accordingly, to explain an individual swimming across the English Channel, or an ordinary individual recovering from a stroke, we cite efficient muscle, powerful lungs and vulnerable brains. Each of these are part of the human body we call organs. But to understand organ physiology, we must descend another level smaller and that is the level of cells. In conventional bio-medical textbooks, cells are the last and thus the ultimate units of life. As a membrane enclosed sac of aqueous solutions, cells are like little soap bubbles, which can be broken but not resolved into still smaller repeating units. So what happened in conventional cell physiology to parts of the living cells like the nucleus, the mitochondria and the Golgi apparatus? They were given away to the department of biochemistry, department of genetics etc. and never came back to conventional cell physiology.

For we know by now that this conventional membrane (pump) view is completely wrong. And I am far from being the first to say this. Max Schultze in his *Protoplasmic Doctrine* said so already in 1861, when he called the cell a lump of protoplasm without a membrane. But as mentioned already on preceding pages, he too was only partly correct.

There are two shortcomings that Schultze and other early proponents of protoplasmic doctrine could not resolve. Again, the time was too early. First, they considered only one kind of protoplasm. Secondly, they could offer only gross *macroscopic* terms and concepts to define that protoplasm. Coming late in time and thus enjoying benefits denied earlier investigators, the association induction-hypothesis has in time corrected both shortcomings as will be shown next.

5.1 Definition of LIFE in terms of microscopic entitles

In the association-induction (AI) hypothesis, the smallest unit of life and hence life's ultimate physical basis, is ***microscopic protoplasm*** or ***nano-protoplasm***. In contrast, the living jelly oozing out of a broken cell like that shown in Figure 1 and once called sarcode or protoplasm is a specific type of ***macroscopic protoplasm***. As a rule, macroscopic protoplasm from one part or region of a living cell, say the cytoplasm, differs from macroscopic protoplasm from another part of the cell like the nucleus or cell membrane. But **each type of macroscopic protoplasm is, without exception, an aggregate of a vast number of the corresponding kind of *nano-protoplasmic units*** (NPU) (Ling 2007a.)

In the AI Hypothesis, life has two facets: being alive and engaging in reversible life activities. At the most fundamental level, being alive signifies the existence of the nano-protoplasm in a low-entropy but stable state called the *resting living state*. Life activities, on the other hand, involve reversible all-or-none shifts between the low-entropy resting living state and the alternative stable high-entropy *active living state*. Figure 15 provides a diagrammatic illustration of the salient features of these fundamental attributes and behavior of a nano-protoplasmic unit.

The low entropy of the nano-protoplasm in the resting living state—illustrated in the right-hand side picture of Figure 15—originates from the near total ***association*** among all the components of the nano-protoplasm: (1) virtually all the water molecules are directly or indirectly adsorbed on the peptide NH and CO groups of the fully-extended protein chains; (2) virtually all the K^+ in the nano-protoplasm are adsorbed on the β-, and γ-carboxyl groups of the nano-protoplasmic proteins and (3) the cardinal adsorbent, ATP

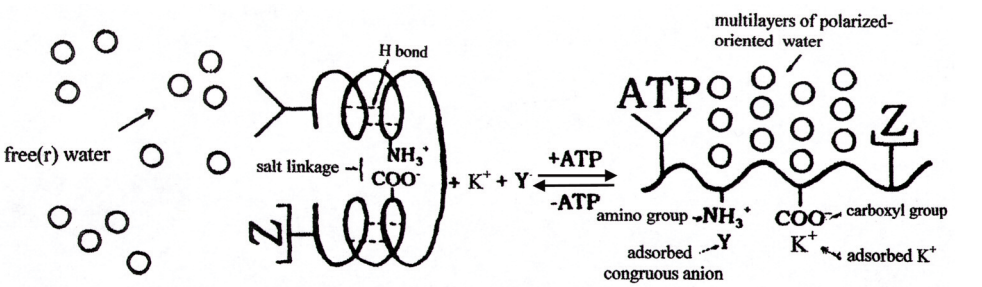

FIGURE 15. Diagrammatic illustration of all-or-none transition of a nano-protoplasmic unit between the fully-extended (resting living) state and the doubly folded (active living) state brought on by the binding onto, or removal of ATP from its specific cardinal site and/or of one or more *auxiliary cardinal adsorbent(s)* including (possibly) Ca^{++} and protein X etc. which are represented together here for simplicity by the single symbol, **Z** rather than by an undefined number of Z's (see Ling 1992 p. 184 including its Figure 8-14.)

is adsorbed on its specific *cardinal site* (Ling 1962 p. iiiv and p. 110.) Auxiliary cardinal adsorbents like that shown as Z in Figure 15 may remain adsorbed on their respective *auxiliary cardinal sites* during the resting living state and the active living state; they also may play a key role in controlling or modifying the shift between the resting and active living state as Ca^{++} and ouabain may do respectively.

For an illustration of the quantitative aspects of a typical nano-protoplasm in terms of molecules and ions, I choose for its exceptional simplicity the cytoplasmic nano-protoplasm of mature human red blood cells (rbc.) A typical rbc assumes the shape of biconcave disk as shown in Figure 16. It has neither nucleus nor other subcellular organelles. 65% of the rbc weight is water. 97% of the remaining 35% of the rbc's weight comprises vast number of copies of a single protein, ferri-hemoglobin (Hb) (Ponder 1948.) It is also known that the content of a rbc does not spill out when the rbc is cut apart (Best and Taylor 1946.) And that at 0° C, the rbc keeps its ATP content better than at higher temperature (Ling and Bohr 1969.) Accordingly, one can use a magic scalpel to cut a single rbc at 0° C into two equal halves and repeat the procedure again and again until at last each of the halves contains just one single Hb molecule. At this time, the content of each half is essentially that of a single rbc cytoplasm nano-protoplasmic unit (NPU.)

Based upon these and the other relevant facts, one can then tentatively represent the nano-protoplasmic unit of the rbc cytoplasm by the formula: $(Hb)_1(H_2O)_{7000}(K^+)_{20}(ATP)_1$. As our knowledge about each nano-protoplasmic unit increases, its formula will change. A general formula for all nano-protoplasm is given in the form of Equation 1, on page 124 of the article, *Nano-protoplasm, the Ultimate Unit of Life* (Ling 2007a.) Assumed to be spherical in shape, the diameter of each NPU of rbc cytoplasm is 8.6 nanometers (nm.) All these pertain to the rbc cytoplasmic nano-protoplasm in its ***resting living state***—as diagrammatically illustrated in the right hand side illustration of Figure 15.

The resting living state is under the pervasive control of the principal electron-withdrawing cardinal adsorbent, (EWC) ATP (in addition possibly to other as yet unidentified auxiliary cardinal adsorbents.) Through the operation of the AI cascade mechanism, the adsorption of ATP on its cardinal site sets in motion the chain reaction so that all the β-, and γ-carboxyl groups on the aspartic and glutamic side chains of the hemoglobin molecule are kept uniformly at a low c-value with preference for K^+ over competing Na^+. And

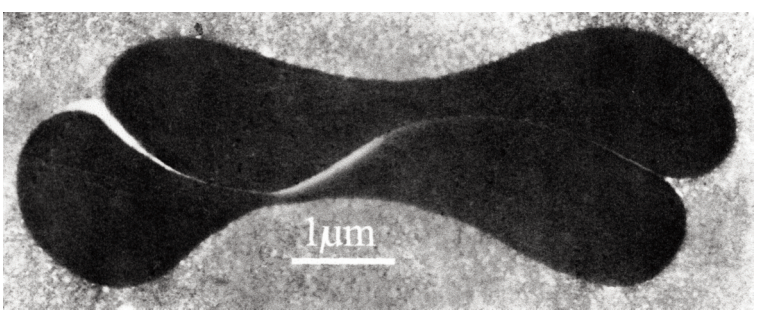

FIGURE 16. Electron micrograph of the cross sections of two mature human red blood cells (in blood plasma.) Cryofixed, freeze-dried and embedded in Lowicryl. (Gift of Dr. Ludwig Edelmann, from Ling 2007a)

all the backbone peptide carbonyl groups are kept uniformly at a low c-value analogue value with preference for multilayer water polarization-orientation.

In contrast, the left-hand side picture of Figure 15 depicts a nano-protoplasmic unit in the ***active living*** *(or* ***dead)*** ***state***, brought on by the removal of the EWC, ATP transiently (or permanently.) As such, the assembly as a whole has high entropy because all the water molecules and K^+ are set free. This follows from the fact that the removal of EWC, ATP sets in motion the AI cascade mechanism leading to a uniform rise of the c-value of all the β-, and γ-carboxyl groups on aspartic and glutamic side chains. And at the high c-value (and with the availability of fixed cations) the β-, and γ-carboxyl groups prefer to engage in the formation of salt-linkages, setting free the adsorbed K^+. Simultaneously, the ATP removal also leads to a uniform decrease of the c-value analogue of the backbone carbonyl groups. At the low c-value analogue, the backbone carbonyl groups prefer to engage in α-helical formation with NH groups of the fourth amino acid residues in both directions along the polypeptide chains. Release of all the multilayers of adsorbed water molecules follows.

Since ATP is the end product of all energy metabolism (Ling 1981) and in cells like the voluntary muscle, there is also a large store of creatine phosphate, which through the action of the enzyme *creatine kinase* maintains a constant level of ATP. Accordingly, the replacement of lost ATP is usually rapid and complete. When for one reason or another, the replacement of ATP fails, the nano-protoplasm will enter the irreversible ***dead state***.

In summary, ***life*** comprises ***being alive*** and ***engaging in life activities***. At the most basic level, *being alive* signifies the existence of nano-protoplasm units in the low entropy *resting living state*, in which all the major components of nano-protoplasm are in direct or indirect association with one another—, an act made possible by the adsorption of the controlling ATP on its specific cardinal site (and possibly adsorption of as yet unverified (auxiliary) cardinal adsorbents also.) The adsorption of the powerful EWC, ATP brings about a uniform decrease of both the c-value of the β-, and γ-carboxyl groups (and K^+ adsorption) and the c-value analogue of the peptide carbonyl oxygen atoms (and multilayer H_2O adsorption) via the AI cascade mechanism. ***Engaging in life activities***, on the other hand, signifies reversible all-or-none (auto-cooperative) shifts between the *resting living state* and the *active living state*. In contrast to the resting living state, the active living state is distinguished by its high entropy in consequence of the liberation of all its K^+ ions and water molecules and the assumption of the nano-protoplasmic protein of the double-folded states through the formation of α-helical intra-polypeptide H-bonds on one hand and the formation of salt linkages between fixed cations and fixed anions on the other.

5.2 Verification of theory on an ultra-simple model

If a theory of life is correct, it would provide the foundation of the basic mechanisms for understanding all living phenomena. Or more correctly, understanding of life at the ultimate lowest level would explain all living manifestations at one level higher and those perceptions would in turn provide the basis for understanding life phenomena at another level still higher—until all living phenomena will be explained—in a way in full harmony with our understanding of the entire Universe comprising both the living and the dead.

To begin with our knowledge at the nano-protoplasmic level, our task is to understand phenomena of cell physiology. The four classical topics of cell physiology are (i) solute (and water) distribution; (ii) solute (and water) permeability; (iii) cellular electrical

potentials; (iv) cell swelling and shrinkage. Progressively more up-to-date explanations of the manifestations of cell physiological activities under each of these four categories have been presented in two books and a more recent lengthy review. They are respectively *A Revolution in the Physiology of the Living Cells* (Ling 1992), *Life at the Cell and Below-Cell Level* (Ling 2001) and *Nano-protoplasm, the Ultimate Unit of Life* (Ling 2007a.)

Other physiological activities of the cell beyond the four categories listed above and their explanations based on prior versions of the AI Hypothesis are found in two earlier books: *A Physical Theory of the Living State: the Association-Induction Hypothesis* (Ling 1962) and *In Search of the Physical Basis of Life* (Ling 1984). In addition, there are also individual review articles on specific subjects including the following: Ling 1977 (energization of biological work performance); Ling 1981 (oxidative phosphorylation & mitochondrial physiology); Ling 1981a, 1990 (active transport across frog skin and other bifacial systems); Ling, Reid and Murphy 1986 (cancer); Ling and Ochsenfeld 1991 (muscle contraction.)

While the interested reader may choose to consult these publications directly, I am going to present below the Abstract of a very special paper by Ling and Ochsenfeld (2008) described immediately below as an illustration of the simplicity and effectiveness of explaining life at one level above nano-protoplasm. The title of the paper reads:

A Historically Significant Study that at Once Disproves the Membrane (Pump) Theory and Confirms that Nano-protoplasm is the Ultimate Physical Basis of Life—Yet so Simple and Low-cost that it Could Easily Be Repeated in Many High School Biology Classrooms Worldwide

Abstract.
In 1889 Abderhalden reported his discovery that there is no (or as shown later, little) sodium ion (Na^+) in human red blood cells even though these cells live in a medium rich in Na^+. History shows that all major theories of the living cell are built around this basic phenomenon seen in all the living cells that have been carefully examined. One of these theories has been steadily evolving but is yet-to-be widely known. Named the association-induction hypothesis (AIH), it has been presented thus far in four books dated 1962, 1984, 1992 and 2001 respectively. In this theory, the low Na^+ in living cells originates from (i) an above-normal molecule-to-molecule interaction (energy) among the bulk-phase cell water molecules, in consequence of (ii) their (self-propagating) polarization-orientation by the backbone NHCO groups of (fully-extended) cell protein(s), when (iii) the protein(s) involved is under the control of the *electron-withdrawing cardinal adsorbe*nt (EWC), ATP. A mature human red blood cell (rbc) has no nucleus, nor other organelle. 64% of the rbc is water; 35% belongs to a single protein, hemoglobin (Hb). This twofold simplicity allows the concoction of an ultra-simple model (USM) of the red blood cell's cytoplasmic protoplasm, which comprises almost entirely of hemoglobin, water, K^+ and ATP. Only in the USM, the ATP has been replaced by an artificial but theoretically authentic EWC, H^+ (given as HCl). To test the theory with the aid of the USM, we filled dialysis sacs with a 40% solution of pure (ferri-) hemoglobin followed by incubating the sacs till equilibrium in solutions containing different amounts of HCl (including zero) but a constant (low) concentration of NaCl. We then determined the

equilibrium ratio of the Na^+ concentration inside the sac over that in the solution outside and refer to this ratio as q_{NaCl}. When no H^+ was added, the q_{NaCl} stayed at unity as predicted by the theory. More important (and also predicted by the theory,) when the right amount of H^+ had been added, q_{NaCl} fell to the 0.1–0.3 range found in living red blood (and other) cells. These and other findings presented confirm the AIH's theory of life at the most basic level: in the *resting living state*, microscopic, or *nano-protoplasm*, is the ultimate physical basis of life. (End of Abstract.) (Figure 17 presented in the main text of the article is transplanted here for emphasis and clarification.)

The following is a list of the highlights presented in the full-length article and their ***new global significance***.

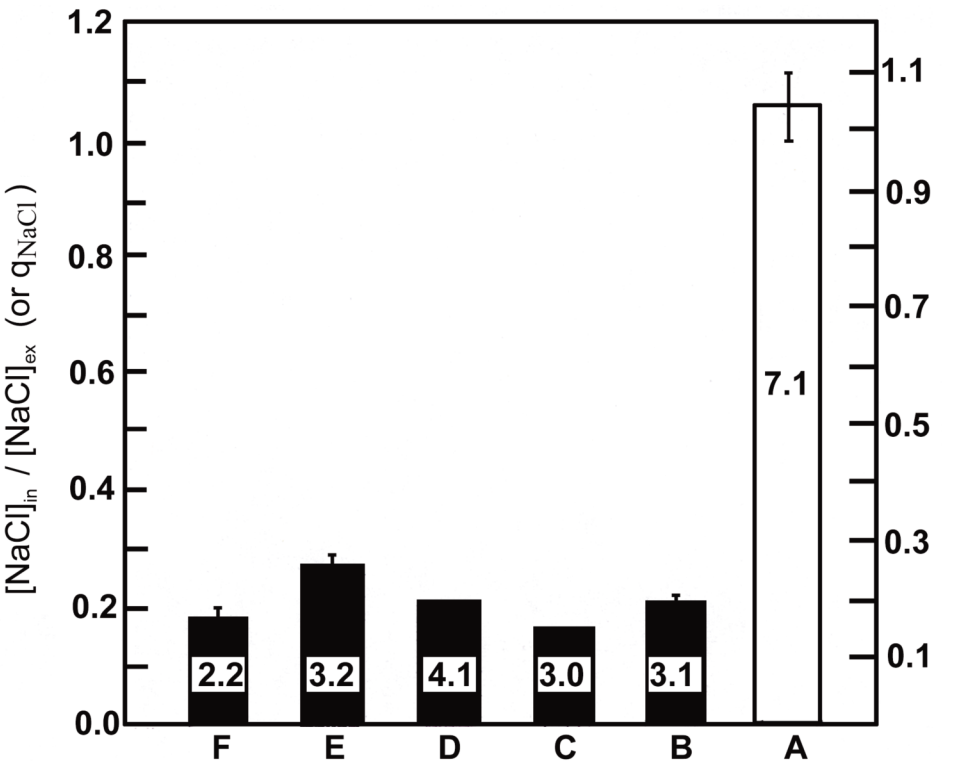

FIGURE 17. The equilibrium distribution ratio of NaCl or q_{NaCl} of ultra-simple models of red blood cell cytoplasmic nano-protoplasm (A) not treated with HCl; (B,C,D,E,F) treated with appropriate amount of HCl. Numbers in bar graphs indicate equilibrium pH of the media bathing that particular set of dialysis sacs. Data were obtained with the aid of two different experimental techniques: equilibrium dialysis studies carried out by M. M. Ochsenfeld and radioactive tracer studies carried out by Dr. Hu Weixiao. (From Ling and Ochsenfeld 2008)

5.2.1 q_{NaCl}

This article presents the first fully successful demonstration in history in a model system of a q value between 0.1 and 0.3 for NaCl at the low (10 mM) concentration range. The bulk of prior work on q_{Na} from my own laboratory and from some other laboratories were based on the use as probes of sodium sulfate or sodium citrate and as a rule at very high concentration. Other studies showed that in those model systems like linear oxygen-containing polymers and NaOH-denatured hemoglobin, the demonstrated q_{NaCl} were as a rule, not far from unity. The low q_{NaCl} profile demonstrated here is shared by most living protoplasm as the theory predicted. The **diversity** of dynamic water structure (all under the heading of polarized-oriented multilayer theory) and their experimental evidence will be introduced by us hopefully not too long from now. Included is the evidence that the dynamic structure of the bulk-phase water in maximally deviated **cancer** cell is different from that in normal cells like muscle for example.

5.2.2 AI cascade mechanism

As many as 500 water molecules have been demonstrated to become "non-solvent" for Na^+ by the attachment of a single H^+ on an H^+-binding site of the hemoglobin molecule. This demonstration confirms the even farther reach of the AI cascade mechanism operating in intact living muscle cells discussed in Section 4.4.4.1 on page 40, where each ATP molecule controls the dynamic structure of an average of 8000 water molecules.

5.2.3 ATP as EWC

The physiological role of ATP as an electron-withdrawing cardinal adsorbent (EWC) has received further confirmation because H^+, its substitute in the present study, is nothing more than a positive charge and hence by definition an EWC.

5.2.4 Chloride binding confirms role as congruous anion adsorbed one-on-one in close-contact in rbc

The extensive adsorption of Cl^- ion on hemoglobin described in detail in the text but not mentioned in the abstract, confirms yet another important theoretical postulation of the AIH: Cl^- is the principle congruous anion of red blood cells (Ling 1992 p. 183; Ling 2001 p. 153.) That Cl^- binding has produced an across-the-board *increase* of the c-value analogue hence the percentage of backbone carbonyl oxygen atoms existing in the α-helical fold conformation—further confirms that the binding of the Cl^- is adsorbed one-on-one in close contact as the theory has predicted.

5.2.5 Explaining Edelmann's LiCl promotion of Cs^+ adsorption

Using laser-microprobe mass-spectrometer analysis (LAMMA), Edelmann discovered that the presence of 100 mM LiCl produced what appears to be a fivefold increase of Cs^+ adsorption on the β-, and γ-carboxyl groups of myosin at the two edges of the A bands of frog muscle (Edelmann 1980.) Like H^+, Li^+ too is a simple and small cation and as such its binding on myosin could in theory produce a qualitatively similar effect on myosin as

that produced by the binding of H^+. Now 100 mM Li^+ is equivalent to a pLi of 1—certainly high enough in concentration to match the H^+ concentration at pH of 2, which produced the minimum physiological q_{NaCl} of 0.1 to 0.3 of the bulk-phase water as shown in Figure 17. In theory, this was accomplished by decreasing wholesale the ***c-value analogue*** of the backbone carbonyl groups. Of course, according to the AIH, a wholesale decrease of the ***c-value analogue*** of the backbone carbonyl groups via the AI cascade mechanism would inevitably be accompanied by a wholesale decrease of the ***c-value*** of the β-, and γ-carboxyl groups simultaneously— but that subject was not pursued in the 2008 Ling & Ochsenfeld study described. Yet what that work did reveal to us led me to the belief that the influence of a high concentration of Li^+ in promoting Cs^+ adsorption is by way of decreasing the c-value of the β-, and γ-carboxyl groups by the adsorption of Li^+. And at the lowered c-value, the adsorption energy of Cs^+ is increased (and the relative binding energy of competing Li^+ decreased) as shown in Figure 12. And a consequence would be what Edelmann had discovered and reported in 1980: increased binding of Cs^+ on the edges of the A bands of frog sartorius muscles. (It bears mentioning that the Cl^- ion that came with Li^+ and Cs^+ will play a similar role as that described for the Cl^- ion that came as HCl in the full-length article of the Ling-Ochsenfeld study.)

5.2.6 Polar NP surface or array of polar NP-NP-NP chains

The NP-NP-NP system of oriented fully extended protein chains with its backbones directly exposed to bulk-phase water have been shown in the (full-length) article to have far-reaching impact on the dynamic structure of the bulk phase water—functionally almost as effective as an NP (or NP-NP system) of a checkerboard of negatively charged N and positively charged P sites uniformly distributed on a two-dimensional surface—which was shown to have the potential of polarizing-orienting water molecules *ad infinitum* under idealized conditions. (For other independent evidence for long-range water polarization-orientation of other NP-NP-NP models, see Ling 2001's Figure 30 and Ling 2006's Figure 14.)

I thank Dr. Raymond Damadian and his Fonar Corporation and its friendly and helpful members for their support, Margaret Ochsenfeld for her skillful cooperation and Michael Guarino, Director of Media and Internet Services, for his patient assistance. Last but not the least, I want to express my deep gratitude to the life-long support from my beloved concert pianist wife, Shirley Wang Ling, who passed away in the summer of 2011 from (incurable) pancreatic cancer.

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