BioImmersion Inc. *Forward Thinking™*

Cruciferous Sprout Complex

Monograph

It has been well established that broccoli, Brussels sprouts, diakon radish and the cruciferous family of vegetables are well researched for their ability to provide anti-cancer protection and to enhance the overall performance of the endogenous antioxidant system. The class of phytonutrient within the cruciferous family responsible for these benefits is the glucosinolates and more specifically the isothiocyantes subclass. Sprouts are one of nature's richest sources of phytonutrients, and by weight are more potent than the mature plant. Globally, sprouts have been used medicinally for thousands of years.

The Cruciferous Sprout Complex is a proprietary, freeze-dried blend of sprouts of Broccoli, Water Cress, Daikon Radish, Alfalfa, Kale, Mustard, Red Clover and Cauliflower. Utilizing Green Technology, seeds that contain high glucosinolate, isothiocyanate and sulforophane content are selected to sprout. After three days of growth the sprouts are arrested at the height of their glucosinolate potential, blanched to kill any pathogenic microbes such as molds, and freeze-dried so as not to destroy any of their heat sensitive nutrients. BioImmersion Green Technology enables the Cruciferous Sprouts to fully manifest their potential as a powerful stimulus for the endogenous production of a class of critically important enzymes known as the Phase II Proteins (P2Ps).

Every cell of our body must have an ample production of P2Ps to successfully cope with oxidative stress both within the cell and outside the cell. Glutathione transferase, heme oxygenase and quinone reductase are three of the approximately 24 phase II enzymes. Collectively these proteins are responsible for neutralizing carcinogen, binding toxins, chelating heavy metals, and reducing free radicals. P2Ps are the enzymes responsible for Phase 2 Liver Detoxification.

Cruciferous Sprout Complex (CSC) was analyzed for isothiocyanate and sulforaphane content. Analytical data shows that the amount of glucosinolates, isothiocyanates and sulforaphanes was 20mg/g, 14.2mg/g and 4mg/g respectively. Other chemical

analyses of CSC showed high content of total amino acids (24%), low concentration of total sugars (1.55%), and low amount of sodium (0.2mg/g). Among amino acids, glutamic acid was found at highest concentration (48mg/g), and histidine at the lowest (8.6mg/g). Calcium, magnesium, phosphorus and potassium were found in amounts of 5.4mg/g, 3.5mg/g, 4.3mg/g and 5.1mg/g respectively. These results represent the unique chemical composition of CSC (see Certificate of Analysis in the Cruciferous Sprouts Dossier).

Oxidation

Every cell has chemical reactions involving the oxidation and reduction of molecules. These reactions or redox pathways, can lead to the production of free radicals. A useful acronym to encapsulate the meaning of the oxidation/reduction process is: OIL RIG (oxidation is losing electrons and reduction is gaining electrons). When a molecule looses electrons, it enters into a state of possessing one or more unpaired electrons and becomes a highly unstable molecule that has electrons available to react with various organic substances. When free radicals react with key organic substrates such as lipids, proteins and DNA, the oxidation can damage and disturb the normal functions and contributes to a variety of disease states.

Many naturally occurring processes can influence the level of free radical production within our body. For example, when cells use oxygen to generate energy, free radicals are created as a consequence of ATP production by the mitochondria. When pathogenic bacteria, yeast and protozoa attempt to invade our bodies they use free radicals as a part of their weaponry. Our immune system counters these assaults by generating its own arsenal of free radicals such as hypochlorus acid to kill the invaders. Exercise, a necessary every day evil, can increase the levels of free radicals. Environmental stimuli such as ionizing radiation (from industry, sun exposure, cosmic rays and medical X-rays), environmental toxins, altered atmospheric conditions (i.e. hypoxia and hyperoxia), ozone and nitrous oxide (primarily from automobile exhaust) generate free radicals. Lifestyle stressors such as cigarette smoking and excessive alcohol consumption are known to affect levels of

free radicals. An overload of psychological stressors can shift the brain into intense metabolic activity thereby generating excessive free radical production. Unhealthy eating patterns are obvious sources for free radicals. In summary, the preponderance of oxidizing agents in our world today, increased psychological stress, poor life style choices and dietary practices are overwhelming our body's ability to neutralize their potential damaging and disease causing effects.

Reactive Oxygen Species (ROS) is a term collectively describing radicals and other non-radical reactive oxygen derivatives. The intermediates may participate in reactions giving rise to free radicals or that are damaging to organic substrates. ROS in living organisms include the following:

Radicals		Non-Radicals	
Hydroxyl	OH.-	Peroxyntirite	ONOO-
Superoxide	O2.	Hypochloric Acid	HOCl
Nitric Oxide	NO2.-	Hydrogen Peroxide	H2O2
Thyl	RS.	Singlet Oxygen	-1O2
Peroxyl	RO2.	Ozone	O3
Lipid Peroxyl	LOO.	Lipid peroxide	LOOH

Reactive Nitrogen Species (RNS) are radical nitrogen-based molecules that can act to facilitate nitrosylation reactions. RNS in living systems include:

Radicals		Non-Radicals	
Nitrous oxide	NO.	Nitrosyl cation	NO+
Peroxynitrite	OONO-	Nitrogen dioxide	NO2.
Peroxynitrous acid	ONOOH	Dinitrogen trioxide	N2O3
Nitroxyl anion	NO-	Nitrous acid	HNO2
Nitryl chloride	NO2Cl		

Many other radical species can be formed by biological reactions, for example: phenolic and other aromatic species are often formed during xenobiotic metabolism as part of natural detoxification mechanisms.

Oxidative Stress

Oxidative stress occurs when the generation of ROS in a system exceeds the system's ability to neutralize and eliminate the free radicals. The imbalance can be the result of disturbance in production of antioxidants, lack of sufficient distribution throughout the body, or by damage to a cell's lipids, protein or DNA, inhibiting normal function. Because of this, oxidative stress has been implicated in a growing list of human diseases as well as in the aging process.

A short list of diseases associated with major types of oxidized molecules

Oxidized Molecule	Disease
oxLDL	Atherogenesis
	Cardiovascular
Protein carbonyls	Alzheimer disease
	Parkinson's disease
Nitroso-compounds	Arthritis
	Insulin Resistance
	Multiple sclerosis
Compounds modified by	Atherosclerosis
Hypochloric acid	Cystic fibrosis
	Colitis

Researchers are making progress in understanding the role of oxidative stress and nitrosative stress in cardiovascular diseases, cancer, diabetes, asthma, inflammatory bowel disease, dermal and ocular inflammation, arthritis and diseases of the central nervous system such as ALS, Alzheimer's, Parkinson's and stroke. This increased awareness of the delicate balance that exists between free radicals and the systems in place to regulate them has given rise to new tools for analysis and corrective therapeutics.

Antioxidant Defense System

A healthy body is equipped with an internal mechanism that is able to cope with the oxidative stressors of the world today; neutralizing, reducing (giving electrons) and generally decreasing inflammation by means of an extensive, highly effective group of protective agents collectively referred to as the Antioxidant Defense System (ADS).

The Antioxidant Defense System includes enzymes and antioxidants to prevent the start of oxidative damage and/or control its spread. There are also enzymes to repair oxidative damage, and mechanisms to target damaged molecules for destruction and replacement. Some essential antioxidants are made in our cells (endogenous antioxidants), and include enzymes such as the Phase II Proteins (P2P), superoxide dismutase (SOD), catalase and glutathione peroxidase; and the small molecules glutathione, carnitine, carnosine, uric acid, coenzyme Q-10 and lipoic acid. Other essential antioxidants such as Vitamin C, E, selenium and certain phytochemicals must be obtained from our diet (exogenous antioxidants). Fruits, vegetables and grains are rich sources of vital antioxidant, abundant with vitamins, minerals and botanicals (phytochemicals). Most of the important antioxidant phytochemicals are derived from the colored part of fruits and vegetables. The terpenes comprise one of the largest classes of phytonutrients. Subclasses of terpenes are the carotenoids, clustered at the yellow-orange-red end of the spectrum, responsible for the golden color of corn, the scarlet in tomatoes, and the orange in carrots. The carotenoids deliver such powerful antioxidants such as the beta-carotene, beta-crpytozanthin, lycopene, lutein and zeaxanthin. Another large class, the phenols, is comprised of the blue, blue-red and violet colorations seen in berries, grapes and purple eggplant. Subclasses of phenols include flavonoids, anthocyanidins, proanthocyanidins and xanthones. Without the antioxidant pigments to save them, plants would quickly die from the suns ultraviolet light and the free radicals generated by photosynthesis. There is much work that has been done and published on the importance of the plant-derived antioxidants relative to human health. Antioxidant research has been performed using many trial models; in vitro models, animal models and human trials. It is well established worldwide that the

ingestion of color-rich raw fruits and vegetables transfers their antioxidant reducing power into the human body.

Objective Markers- From ORAC to Functional Bioavailability

The scientific standard for measuring the antioxidant potency of foods is currently expressed in ORAC units (Oxygen Radical Absorbent Capacity). The ORAC assay measures the total antioxidant capacity of a product. A high ORAC score indicates a high total antioxidant capacity. As part of the selection process of a particular fruit or vegetable, the ORAC value is an important consideration. For example, in the selection process for the BioImmersion blueberry, we choose the variety that has the highest ORAC score of all berry, genus and species, in North America (tests comparisons done by the USDA); the wild blueberry grown in the coastal fields and barrens of northern Maine and eastern Canada. The Nova Scotia wild blueberries have an unusual diversity of anthocyanin molecules, from 25 to 30 as compared to the 3 to 5 with other berries. The six phenolic rings of each anthocyanin molecule have the capacity to absorb energy coming from free radicals (neutralizing free radicals), while the anthocyanin itself is changed very little and continues to function for further antioxidant work.

ORAC values are a useful tool, and yet give us only a part of the picture, as the question of bioavailability comes into play. Further measurements and markers must be looked at. For example, after consuming one capsule of BioImmersion pure Wild Blueberry Extract (equivalent to ingesting one and one-quarter cups of wild blueberries), one would expect blood ORAC values to increase. Bioavailability is determined by measuring post-pranial ORAC blood values. Additionally, increasing the antioxidant power of the blood should enable one to lower certain oxidized blood molecules such as oxLDL, a very dangerous free radical. Therefore, measurements are taken of oxLDL levels before and after consumption of the Wild Blueberry Extract. The measurement of changes in certain blood proteins, serving as an indicator of effectiveness for a given BioImmersion Therapeutic Foods, is termed Functional Bioavailability Testing. Another marker that our collaborating scientists

analyze for is called 8-isoprostane. This marker is reflective of the general level of lipid oxidation throughout the body. There are certain inflammatory cytokine and chemokine levels that should be analyzed to determine the functional bioavailability of the Wild Blueberry, the pigments of blueberries do not only deliver antioxidant activity but also have anti-inflammatory capacity. Therefore, blood levels of anti-inflammatory markers IL-18, MCP-1, COX-2 and the transcriptional molecules Nf-kappa B, are analyzed.

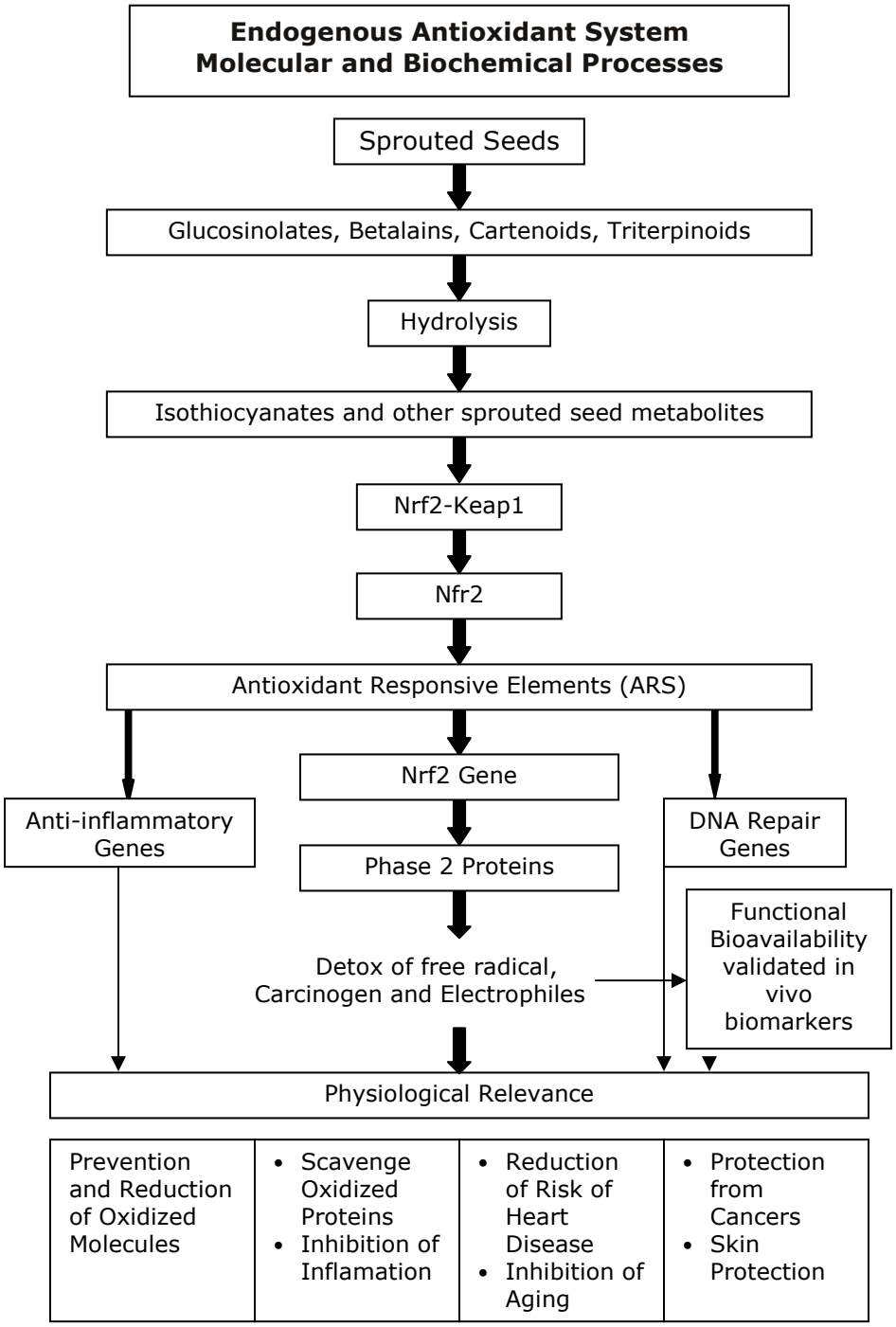
Endogenous Antioxidant Production

There are yet other sets of markers used for determining Functional Bioavailability. The markers our scientists employ in evaluating the endogenous antioxidant power of the Cruciferous Sprouts Complex. The cruciferous vegetables, which stimulate the augmentation of endogenous antioxidant power within the Antioxidant Defense System, do so without having any significant ORAC values. Among the most powerful and important of these antioxidants are the Phase II Proteins (also called Phase II Enzymes or P2Ps). Glutathione transferase, heme oxigenase, quinone reductase, epoxide hydrolase, UDP-glucuronosyl transferases and gamma-glutamylcysteine synthertase represent six out of twenty-four of the body's P2Ps; all play key roles in the health of every cell in our body, scavenging and inactivating harmful chemicals, carcinogens and neutralizing free radicals and electrophiles. They are particularly plentiful in liver cells, responsible for phase II liver detoxification.

The phytochemicals within the cruciferous family responsible for its antioxidant inducing power are the glucosinolates. The glucosinolates are a big family with three branches, one being the isothiocyanates. Within the isothiocyanates there are 300 to 400 different compounds, one being the sulforophanes. It is the isothiocyanates and the sulforophanes in particular that are known to be the key initiators for a chain reaction of events occurring within human cells that leads to the genomic transcription of Phase II Enzymes. This endogenous system is driven by a group of genes controlled by "internal switches" called the Antioxidant Responsive Elements (ARE). ARE's produce the powerful P2P antioxidant enzymes.

Nrf2-Keap ARE

The process of P2P activation involves Nrf2-Keap1, a complex interaction of two cytoplasmic proteins that reside in every body cell. This system acts as a sensor to detect the presence of oxidative compounds. When ROS insult is detected, Nrf2 is released from these complexes, and binds to specific regulatory areas (the ARE) on the resident P2P genes. Generally, various ARE-driven genes play important roles in human health. These genes engage in the coding of DNA repair enzymes and in the management of inflammation. P2P and Nrf2-ARE regulatory system and their coded proteins are important in the cytoprotection of cells, genetic material and more. Recently published studies have shown that P2P plays an important role in the protection of neural cells, stem cells, and endothielial cells. The isothiocyanates and sulforophanes from cruciferous vegetables activate this system. The activation of P2P is crucial for prevention of conditions associated with extended oxidative stress.



All together this is the machinery that controls our endogenous antioxidant production. It is well known in animal experimentation that if this system is down the animals become very sick, developing many types of cancers, aging rapidly (so much so that their life span is 20% of a normal animal). The evidence shows that

the P2P genes are very important for longevity and that it is imperative to maintain the function of P2P genes at a high level.

Functional Bioavailability: Measuring the Effect of Pytonutrients

As mentioned earlier, many parameters are looked at for determining the value of a particular plant material in a product. Examination of markers that will determine the functional bioavailability of the isothiocyanates within cruciferous vegetables is as follows: The first consideration for any ingested product is that it obviously has to come into the body without harm. Secondly, the bio-nutrient has to go into the blood stream and from the blood stream to the whole body to maintain its distribution in the tissues. We validate these prerequisites indirectly through the process of functional bioavailability testing. In analyzing for functional bioavailability one eliminates the concern of absolutely knowing how much of a substance gets into circulation, clears the liver and is delivered into the tissues. Functional Bioavailability looks at certain target enzymes in the blood; enzymes that can easily be measured and have been linked to chronic disease states; levels of oxLDL, P2P, PON-1, CETP, QR1 and sEH are examples of such enzymes. Many of these target enzymes are of intense interest to pharmaceutical companies for drug development.

Utilizing a two-step methodology, ingredients are initially subjected to ex vivo evaluation in sera for ability to affect target enzyme levels. Next, successful candidate materials are tested in an in vivo, acute effect clinical case study evaluation in humans. This is a relatively non-invasive procedure generally accomplished with blood from a finger. The following are protein/enzyme markers that we presently measure.

QR1 and P2P

Quinone Redutase-1 (QR1), one of the P2P group, is commonly used to measure materials ability to induce P2Ps. QR is an enzyme found in human cells that reduces quinines in the body and protects cells against the toxic effects of free radicals and oxygen species. Quinones are compounds found in plants that enter the body

through food. These compounds are toxic and can lead to susceptibility to chronic diseases and even cancers.

oxLDL

The diagnostic and predictive value of oxLDL levels has been intensively investigated. Published studies indicate that oxLDL is predictive of atherogenic conditions and heart disease. Studies on young healthy volunteers showed that individuals with high levels of oxLDL had increased risk to develop cardiovascular diseases. Other studies showed that oxLDL could also be involved in the development of insulin resistance. These and numerous other reports provide justification for the maintenance of low oxLDL levels in the blood.

PCO

Clinical studies have suggested that increased levels of protein carbonyls (PCOs) are associated with Alzheimer's disease, chronic lung disease, chronic lung failure, cystic fibrosis, diabetes and rheumatoid arthritis. Damaged proteins generated by direct oxidative attack on lysine, arginine, proline, threonine, or by secondary reactions of cysteine, histidine or lysine residues with reactive carbonyl compound (RCOs), can lead to the formation of protein carbonyl derivatives. Specific protein carbonyls cannot be easily differentiated and identified through the use of simple chemical analyses; consequently, protein carbonyl totals are used as markers of protein oxidation. Carbonyl compounds are relatively more difficult to induce than other oxidized molecules, and are therefore considered to indicate a more severe condition of oxidative stress.

HDL

Numerous epidemiological studies have found an increased risk of fatal and non-fatal coronary heart disease events being associated with a low concentration of HDL-Cholesterol in the bloodstream. HDL is involved in antioxdative effects, preservation of the endothelial function, and protection of cells and organs. Therefore, it is important to maintain proper blood levels of HDL throughout the lifespan.

CETP

Cholesteryl Ester Transfer Protein (CETP) is an enzyme secreted by the liver that is eventually absorbed into the blood. Once in the blood, it acts as a regulator of "reverse cholesterol transport" by modulating cholesterol activity through the transfer of triglycerides from LDL to HDL particles. Cardiovascular disease can develop as a result of an accumulation of LDL cholesterol. Malfunction of transfer proteins leads to higher CETP levels and an overabundance of LDL. Published research reports an inverse correlation between CETP and HDL blood levels.

PON-1

Paraoxonase 1 (PON1) is a human blood enzyme directly associated with maintenance of healthy HDL levels. PON1 is known to modulate the metabolism of lipids, an important factor in the onset of cardiovascular disease. Increased PON1 activity is also associated with a reduced accumulation of oxLDL. PON1 further influences cardiovascular health by inhibiting the overload of lipids that leave fatty streaks on arterial walls. A recent genetic study has also suggested that PON1 gene expression is important for healthy aging. Simulation of PON1 activity provides important health benefits.

sEH

Soluble Epoxide Hydrolase (sEH) is a pharmaceutical research target for treatment of end organ damage associated with cardiovascular and renal diseases. This is due to the fact that decreased renal epoxygenase levels are associated with development of hypertension. sEH is a blood enzyme that is responsible for converting expoxyeicosatrienoic acids (EET) into dihydroxyeicosatrienoic acids (DHET). EET are acids that have antihypertensive and anti-inflammatory properties. They are also important in maintaining renal vascular function. DHET is a converted form of EET. When epoxides are hydrated they form corresponding diols, which have been attributed to many inflammatory disorders, including adult respiratory distress syndrome. sEH inhibition could affect hypertension, inflammation, and vascular smooth muscle cell proliferation. sEH inhibition also may combat the damage of angiotensin on healthy artery function. Animal studies have shown that regulation of sEH promotes healthy blood pressure levels. sEH disruption in male animals resulted

in a decrease in blood pressure, suggesting the sEH could offer promise. Research on lung inflammation due to tobacco smoke has shown that sEH was involved in the metabolism of chemical mediators that contribute to reduced inflammation. Lowering sEH blood levels is obviously very important.

Clinical Trials and Concluding Remarks

Numerous trials have been performed on the BioImmersion Cruciferous Sprout Complex. Trial participants consumed 2 tsp per day of the Cruciferous Sprout Complex over a 20 day period, with positive changes observed in the Functional Bio-Availability markers: average P2P increased 2.6 fold (measure in liver cells); average reduction in oxLDL levels was -69%; average increase HDL 30%; average reduction in 8- isoprostanes and total peroxides were -25% and -20% respectively; CETP levels dropped by 50%; and PON-1 levels increase by an average of 18%.

The initial trials indicate the tremendous importance of the Cruciferous Sprouts for the enhanced production of the critically vital endogenous antioxidants. We look forward to the results of future trials as they are completed in the field with practicing doctors and at the bench with our collaborating scientists, worldwide. As with all of our products this is a never-ending phase of the work.

The Cruciferous Sprout Complex should be strongly considered as a foundational part of a systematic program to arm the body with the necessary antioxidant power to overcome the oxidative assault generated in our environment today from the rising tide of chemicals, toxins and increasingly virulent pathogens. A diligent multi-faceted nutritional approach can regenerate the body and bring healing and balance. First, the Cruciferous Sprout Complex taken several times a week as a stimulus for the body endogenous antioxidant production and for the liver's ability to detoxify. Second, the consumption of the Wild Blueberry Daily, the High ORAC Synbiotic Formula and/or the No. 7 Systemic Booster, bring on board berries and fruit powerful exogenous antioxidant support. Third, the regular consumption of the various BioImmersion Synbiotic Formulas, such as the High ORAC and the No. 7 is

imperative to protect the GI tract from oxidative assault generated by food borne toxins and pathogens.

Governments worldwide recognize the devastating results of the fast food culture. Diseases of oxidative stress such as cancer, heart disease and diabetes are rampant. Globally, the 5 to 9 Fruits and Vegetables a Day Campaign encourages the citizenry to bring back into their diet these critical antioxidants and antioxidant generating power.

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