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Opinion paper

Physics and the molecular revolution in plant biology: union needed for managing the future

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Abstract: The question was asked if there is still a prominent role of biophysics in plant biology in an age when molecular biology appears to be dominating. Mathematical formation of theory is essential in systems biology, and mathematics is more inherent in biophysics than in molecular biology. A survey is made identifying and briefly characterizing fields of plant biology where approaches of biophysics remain essential. In transport at membranes electrophysiology and thermodynamics are biophysical topics. Water is a special molecule. Its transport follows the physical laws of osmosis and gradients of water potential on the background of physics of hydraulic architecture. Photobiology needs understanding of the physics of electro-magnetic radiation of quantitative nature in photosynthesis and of qualitative nature in perception by the photo-sensors cryptochromes, phototropins and phytochrome in environmental responses and development. Biophysical oscillators can play a role in biological timing by the circadian clock. Integration in the self-organization of modules, such as roots, stems and leaves, for the emergence of whole plants as unitary organisms needs storage and transport of information where physical modes of signaling are essential with cross talks between electrical and hydraulic signals and with chemical signals. Examples are gravitropism and root-shoot interactions in water relations. All of these facets of plant biophysics overlie plant molecular biology and exchange with it. It is advocated that a union of approaches of plant molecular biology and biophysics needs to be cultivated. In many cases it is already operative. In bionics biophysics is producing output for practical applications linking biology with technology. Biomimetic engineering intrinsically uses physical approaches. An extreme biophysical perspective is looking out for life in space. Sustained and increased practice of biophysics with teaching and research deserves strong encouragement.

Keywords: bionics; clock; development; electro-physiology; photobiology; photosynthesis; self-organization; signaling; water relations

1. The Molecular Revolution, Systems Biology and Biophysics

Molecular biology based on remarkable advances in analytical techniques was a fundamental breakthrough in the last part of the 20th century. It has advanced biology to an extent that it does not appear exaggerated to speak of a molecular revolution. However, as it is not unusual in the history of science with new breakthrough technologies, it has thence dominated biology, including plant biology. The development of analytics with raising the demand for increasingly effective machines has led to the capacity of analytically realizing complete sets of molecular contents of living cells and organisms, such as their genes (genomics), gene activities (transcriptomics), proteins (proteomics), metabolites (metabolomics), and any other "omics". Documenting these contents has been taken to mean getting hold of entire systems, and the term systems biology was coined. However, these lists of inventories are rather descriptive. Quantitative biology should not fall behind and theory as it is intrinsic in biophysics is needed. A union of approaches needs to be cultivated.

Bioinformatics is required for handling the enormous sets of data of the omics. Conversely, biomathematics is approaching theoretical understanding. What is a system in biology? The various compounds of the omics are modules. As such they reveal little about the structure and function of complex living systems. As already Aristotle recognized, the whole is much more than the sum of its parts. Integration of modules by self organization leads to emergence of systems with completely new properties [42,51,52]. Certainly, the knowledge of the modules is very important. However, the phenomenon of emergence tells us that we must not restrict ourselves to the pure modularity. It is now increasingly recognized that a new view on systems biology is needed which biomathematics is developing [35,36,37,54]. Physics is more disposed to mathematics and theory and biophysics rather than molecular biology can introduce this into plant biology.

Realizing that we need both, the understanding of the modules and their integration in self-organized systems, it is evident, that a union is needed for mastering future challenges. Biophysics is essential in training and research in plant biology for fulfilling the needs of further basic understanding of plant life and the practical implications given by the enormous services made by green organisms as primary producers for the existence of life on Earth. A sequence of examples is used here in an overview for developing the perspective of a union of biophysics with molecular assessments and showing where such interdisciplinary approaches are already fostering essential advances for the future. These are transport of mineral ions, water and metabolites, photobiology, development and signaling, biological timing, self-organization, and bionics, in all of which biophysical processes are inherently indispensable (Figure 1).

2. Plant Transport Physiology

Transport is the basis of integration processes for the emergence of complex plant systems [53]. It is mediating resource allocation and distribution to subsystems. It carries signaling. Perhaps together with photobiology transport physiology is one of the two foremost topics in plant biology with implications of biophysics.

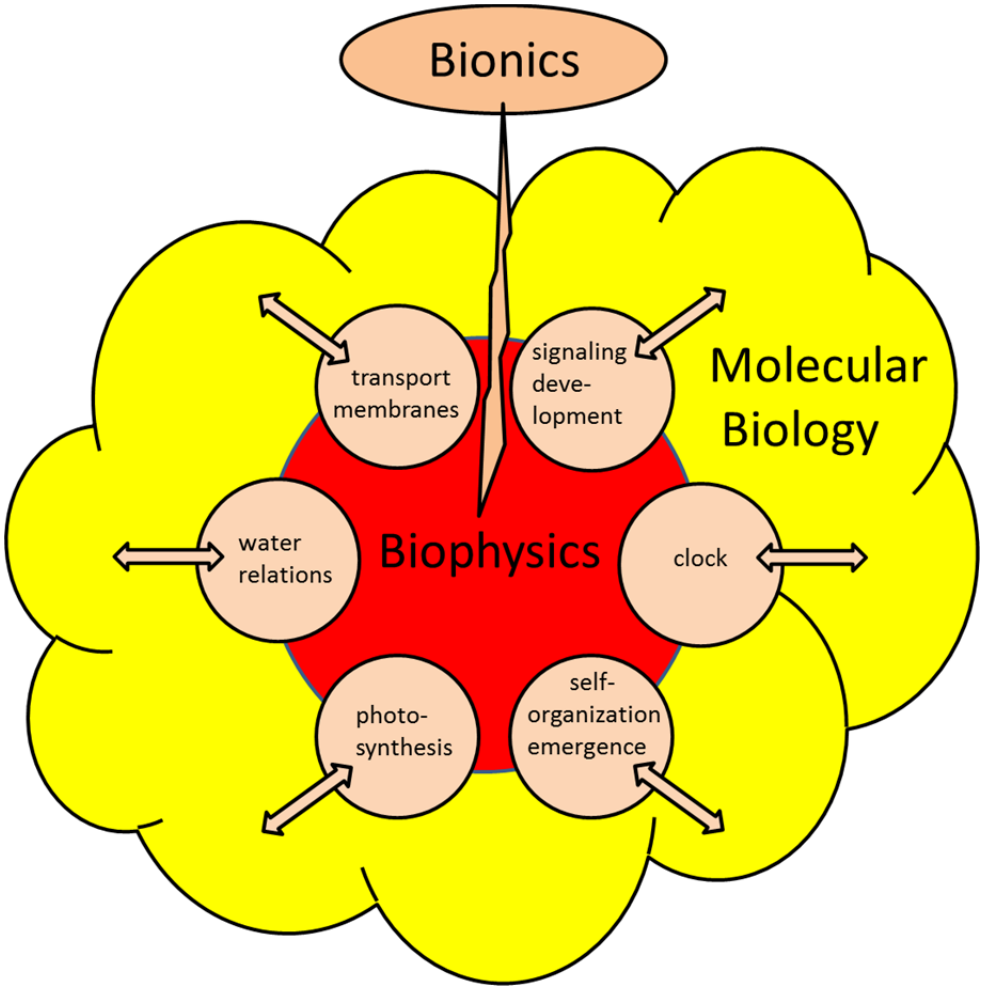


Figure 1. Facets of plant biophysics overlaid by plant molecular biology and exchanging with it, and producing output for practical applications in bionics.

2.1. Thermodynamics and the biophysics of gradients

Transport across membranes is biophysically controlled by gradients between the two sides of membranes, viz. chemical gradients for electrically neutral solutes and electro-chemical gradients for electrically charged ions. Transport along chemical concentration gradients is governed by Fick's law:

$$\frac{dQ}{dt} = -D \times A \times \frac{dc}{dx} \tag{1}$$

An amount of substance, dQ, diffuses during an amount of time, dt. A is the cross sectional area across which diffusion occurs. The concentration difference, dc, along a coordinate dx is the driving force. D is a diffusion constant. For ions deduced from thermodynamics the Nernst-equation for equilibrium and the Goldman-equation for constant electric field show quantification with gradients of concentrations or chemical activities (a) and electric potential (ΔE) across membranes [103]:

$$\text{Nernst-equation } \Delta E = -\frac{RT}{zF} \times \ln \frac{a_a}{a_b} \tag{2}$$

where R is the universal gas constant, F the Faraday constant, z the electric ion charge and T the absolute temperature (Kelvin), subscripts a and b indicate different compartments separated by a membrane;

Goldman-equation written for three univalent ions (z = ±1)

$$E = \frac{RT}{F} \ln \left(\frac{[K_i^+] \cdot P_K + [Na_i^+] \cdot P_{Na} + [Cl_o^-] \cdot P_{Cl}}{[K_o^+] \cdot P_K + [Na_o^+] \cdot P_{Na} + [Cl_i^-] \cdot P_{Cl}} \right) \quad (3)$$

where ion concentrations or activities are in [], subscripts o and i refer to two sides of a membrane (e.g. outside and inside compartment) and P are the permeability coefficients for the various ions as indicated by the subscripts of element symbols.

The technical challenges right from the beginning were the chemical measurements of ion concentrations and electrophysiological measurements of membrane potential, chemistry and physics going hand in hand. The origin of plant electrophysiology and with it biophysics was possibly in 1872 when J. Burdon-Sanderson [16] measured the first action potentials of plants in the Venus' flytrap *Dionaea muscipula* after Charles Darwin had suggested it and sent him some specimen of the plant. Burdon-Sanderson used extracellular electrodes. The first recordings of electric membrane potentials of plants with intracellular electrodes were obtained only 60 years later by Umrath [101] using the large internodial cells of charophytes which allowed impalement with micro-electrodes. Further progress was obtained with other giant algal cells in addition to charophytes, e.g. *Acetabularia, Valonia, Hydrodictyon* and others [80] and only still 30 year later in the laboratory of Noë Higinbotham the first intracellular recordings of cells of higher plants were obtained [22].

Sampling for direct analysis of contents of intracellular compartments except for the vacuoles of giant algal cells was impossible. Compartmentation analysis was introduced measuring exchange of ions between tissue pre-labeled in a radioactive ion solution up to establishment of kinetic equilibrium of influx and efflux and a non-labeled solution otherwise identical to the labeling solution. A complex set of equations was developed relating fluxes and pools, i.e. for assessing the individual fluxes at the plasma membrane and tonoplast and the ion contents of cytoplasm and vacuole [78,104].

It is important to realize that now, in the age of molecular "transportomics", the compartmentation analysis with measuring kinetics of efflux of the labeled ions in exchange for the non-labeled ones are still used to biophysically quantify ionic relations [1,14,41]. Currently a huge number of membrane transporter molecules are qualitatively identified and characterized. However, molecular biology does not tell us anything about their quantitative operations, while conversely thermodynamics do not tell us anything about the nature of the molecular hardware involved. Both need each other.

Individual fluxes by specific ion transporters in complex functions of cells and whole plants are usually interconnected in functional networks and their feed-back and feed-forward dynamics. An outstanding example is the control of the opening and closing, respectively, of stomatal pores by guard cells, which is essential for the life of plants by fine-tuning their performance in the ecological starvation/desiccation dilemma of CO_2-uptake and H_2O-vapor loss, respectively. It is a biophysical problem at two different levels, namely both gas exchange and ion-transporter networks. A remarkable mathematical model for functioning of the latter has been presented by [19].

2.2. Free energy of molecular pumps and biophysics of activity gradients of solutes

With thermodynamics biophysics is much involved in the debate of distinguishing passive and active transport. Passive transport is driven by existing chemical gradients of non-charged solutes or electro-chemical gradients of ions and cannot operate beyond thermodynamic equilibrium. Conversely active transport is uphill. A vigorous debate had occurred in the 1970ies over what that should imply in steady state equilibrium thermodynamics or in non-steady state dynamic irreversible thermodynamics [103]. Strictly spoken active transport had to be considered where the transport of molecules or ions (for simplicity called "particles") was directly driven by the source of metabolic energy. This was assumed to be mediated by so-called pumps, mostly ion pumps. It was termed primary active transport because it was realized that there were many uphill transports not directly linked to the ultimate metabolic energy source. These were brought about by flux couplings where the gradient of a particle *a* drove a flux of that particle which was coupled to the flux of a particle *b* which allowed the transport of particle *b* against its gradient. This was termed secondary active transport.

The concept matured to acceptance by the molecular identification of membrane proteins which functioned in primary active transport, mostly of ions. There had been the hypothesis that some primary transport processes could be directly coupled to the energy of electron transport and the redox-energy of mitochondria [44–47] or the thylakoids of chloroplasts [57,79], however, it turned out that the primary energy source was almost always ATP [53]. Proton-pumps, or H^+-ATPases, and calcium-pumps, or Ca^{2+}-ATPases, were identified at various membranes in plant cells, i.e. the plasma membrane and the tonoplast and others, and characterized in detail by methods of molecular biology. At the tonoplast an additional proton-pump using the free energy of inorganic pyrophosphate was detected, the H^+-PPase. ABC transporters (from ATP-binding casette) are pumps directly using ATP for the transport of other solutes, e.g. glutathion-metal complexes.

The free energy available by the hydrolysis of ATP is given by

$$\Delta G_{ATP} = \Delta G^0_{ATP} + RT \ln \frac{[ADP]+[P_i]}{[ATP]} \quad (4)$$

where ΔG^0_{ATP} is the energy under standard conditions, concentrations are in [] and P_i is inorganic phosphate. Ion pumps establish concentration gradients and electric potentials across membranes. They are electrogenic. The electrochemical ion gradient $\Delta\mu$, across a membrane separating two compartments, e.g. 1 and 2, for example for protons $\Delta\mu_{H+}$, is given by

$$\Delta\mu_{H+} = z\,F\,\Delta E_{2\text{-}1} + RT \ln \frac{c_1}{c_2} \quad (5)$$

Biophysical assessments of such relations mark the limits within which the molecular entities of the various pumps can operate. An H^+-ATPase thermodynamically can only achieve net electrogenic H^+ pumping at $\Delta\mu_{H+} \leq \Delta G_{ATP}$.

2.3. Transporter molecules: Setting up molecular biophysics of membranes

Molecular biology is currently providing the isolation and molecular characterization of a plethora of transporter molecules in membranes, i.e. carriers and ion channels. Among ion carriers there are cation exchangers, e.g. Na^+/H^+ and Na^+/K^+ for maintenance of Na^+-K^+-homeostasis. Among ion channels there are families of cation (K^+, Ca^{2+}) and anion (NO_3^-, Cl^-, $malate^{2-}$) channels [30]. One can speak of channelomics.

Techniques of molecular biology allow handling of transporter molecules, but we realize that this can only be documented and followed functionally by applying biophysical measurements:

- Messenger RNA (mRNA) and complementary DNA (cDNA) can be isolated and cloned. By introduction into host cells they can be expressed in recipients where they normally do not occur. Such heterologous expression, often in the oocytes of the frog *Xenopus laevis*, then allows studying their specific functions.
- Using gene technology transporter proteins in living plant cells can be labeled. A green fluorescent protein (GFP) is attached. Using fluorescence-optical techniques in confocal microscopy this allows sub-cellular spatio-temporal localization of the labeled proteins including specific transporter molecules in specific membrane sites.
- Transporter molecules can be modified by molecular engineering. Particular sequences of amino acids or individual amino acids of transporter proteins can be exchanged for others. In this way structure-function relationships can be assessed at the level of molecular fine-structure.

Techniques of biophysics for functional assessment of the products of molecular biology can be the traditional ones already described above. Even surface electrodes are still important devices, e.g. relating action potentials of *Dionaea muscipula* to the subsequent molecular and physiological processes at the levels of genes, enzyme proteins and resorption of solutes from the prey. This is a nice example of biophysics integrated with molecular biology [9]. However, a biophysical breakthrough was in 1976 the development of the patch-clamp technique by E. Neher and B. Sakmann (Nobel Prize 1991) [71,72,81] originally for animal cells and in 1984 with the first measurements in plant cells [85].

- A small piece of a membrane ("patch") is attached to the opening of a micro-pipette with an orientation of the natural inside out or outside out, or a whole cell can be attached. Both sides of the membrane become accessible for experimental manipulation in biophysical studies, e.g. of solute concentrations at both sides of the membrane and of electrical gradients across the membrane. In plants it was important to isolate protoplasts from the cell wall to give the mouth of the micro-pipette access for measurements at the plasma membrane [66] and to use isolated vacuoles for measurements at the tonoplast [31,32,85].
- For groups of ion-channels and even for single channels the relationship between electrical current flowing across the membrane and the electrical potential across the membrane (voltage) can be characterized measuring the current/voltage relationships under the various experimental conditions chosen.
- Some channels can be passed in both directions. Often they are one way roads only in one direction, i.e. there is rectification outside-in or inside-out, which is an essential property revealed in patch-clamp measurements.

- Channels are not open continuously. There is gating where channels show a certain opening and closing probability. These dynamics are essential for functioning of channels. Patch-clamp measurements reveal these relationships.

Measuring trans-membrane electrical potentials with micro-electrodes provides information different from the types of information given by the patch clamp technique and is still needed, e.g. in studies of signaling and action potentials. However, the break through with the patch clamp technique and the revolution of molecular genetics which emerged somehow simultaneously joined in a union of biophysics and molecular biology fostering scientific advances now and in the future. Much information is currently obtained on the molecular structure of ion channels [30]. However, quantitative assessment of individual ion fluxes remains essential. This is accomplished by compartmentation analysis (Section 2.1). Another particularly biophysical advancement for the quantification of ion fluxes was the development of ion-selective microelectrodes, e.g. for protons, potassium, sodium, ammonium, calcium, chloride and nitrate, applied to the measurement of solute ion uptake in a variety of plant organs under various conditions [73]. The molecular expression of the gene products of transporters must be verified at the functional level. Specific molecules and protein complexes of channels and transporters must be evaluated jointly by molecular biology and the biophysical electro-physiology. The current progress is documented in a detailed and remarkably comprehensive review by Hedrich [30]. This links membrane transporters to functioning of entire cells and whole-plant behavior [30,73].

2.4. Vision in green cells: the eye of Chlamydomonas

We shall deal with the role of biophysics in photobiology below. However, at this stage it appears the best place to mention vision in green algal cells [33] with far reaching perspectives given by the union of molecular biology and patch-clamp biophysics.

During motion of cells of the unicellular flagellate alga *Chlamydomonas reinhardtii* rotating around their cell axis the orange red eye spot periodically darkens the photoreceptor which allows perception of the direction of light. The eye spot consists of a highly ordered array of carotenoid vesicles. It is not the actual eye of the alga. This is the photoreceptor in the plasma membrane which is a proton channel coupled to the chromophore rhodopsin, related to the retinal of our own eyes and named channel-rhodopsin. Its molecular structure is well characterized. Heterologous exprimation and patch clamp measurements have shown that light triggers a change of the channel from the closed to the opened state. This elicits three currents, a first current activity of the receptor channel and a faster and slower subsequent current which mediate coupling to the machinery of flagella movement. The photoreceptor is a unique light-activated H^+ ion channel functioning as a molecular light-switch [69]. It has received considerable interest in neurobiology because with heterologous exprimation in neurons it allows to use the light activation for studying complex switching between neurons [11].

2.5. A conclusion: Continuity of plant-membrane biophysics from 1872 into the age of molecular biology

Membrane transport was at the beginning of plant biophysics with a continuity in the development of electro-physiology by the first extracellular measurements (1872), the first trans-

membrane measurements in giant algal cells (1930) and in cells of higher plants (1960) followed by the patch-clamp technique (1976), which now puts biophysics in the core of modern membrane research at the level of molecular dimensions.

2.6. Transport of water: H_2O a special molecule

Basically the same rules apply to the transport of water molecules as to the solutes. Nevertheless as the major solvent water is a special case in life. Specific channels for the transport of water across membranes are the aquaporins [60,61], which molecular biology can isolate, characterize and engineer like the other channels discussed above. However, due to the essential role of water in all organisms and very conspicuously in plants water transport is a particular domain of biophysics [75].

At the cell level the Pfeffer-cell developed by Wilhelm Pfeffer (1877) is describing the distribution of water across a membrane:

$$\Delta\Psi = \Delta P - \sigma\Delta\pi \tag{6}$$

Ψ is the water potential, as a key parameter in all water relations; $\Delta\Psi$ is the water potential gradient, ΔP is the gradient of hydrostatic pressure, which in plant cells is called turgor pressure. $\Delta\pi$ is the gradient of osmotic pressure given by concentrations of solutes. The cellular process of water transport driven by $\Delta\pi$ is called osmosis. The coefficient σ having values between 0 and 1 is characterizing membrane permeability. If the membrane is strictly semi-permeable, i.e. fully permeable for the water molecules and impermeable for solute molecules $\sigma = 1$. If the membrane also has non-restricted permeability for the solute molecules $\sigma = 0$. The water potential of a solution is given by the difference of the chemical activity of the water in the solution μ_{H2O} and the chemical activity of pure water μ^0_{H2O}:

$$\Psi_{solution} = \frac{\mu_{H2O} - \mu^0_{H2O}}{V_{H2O}} \tag{7}$$

where V_{H2O} is the partial molar volume of water.

Physical methods are used for determining the parameters. Often π is measured cryoscopically. Ψ of leaves and shoot systems has been measured with the Scholander pressure chamber [82,99], where the organs are placed inside the chamber and the cut ends of shoots or petioles stick out through a tight seal. A gas pressure is applied to the chamber and the equilibrium pressure with the atmosphere where water is just starting to come out of the xylem vessels is taken to be equivalent to the water potentials of the organs. A more precise apparatus is the intracellular pressure probe [34,96], where an oil filled capillary is inserted into cells and the cellular turgor-pressure can be measured directly. As a xylem pressure probe the device can also be inserted into xylem vessels [65,106] or it can be attached to the cut ends of roots as a root pressure probe [65,95,108]. It allows measuring pressures and deduced variables including Ψ by using equation (6) under a wide range of experimental conditions.

At the whole plant level molecular labeling of aquaporins has been applied to show their involvement in water transport. Molecular transformation with the gene for luciferase [39] can be used as a reporter gene for the activity of the promoter of the aquaporin so that the intensity of bioluminescence elicited shows the spatiotemporal activity of the aquaporin [88]. Such molecular engineering allows conclusions about whole plant performance. Beyond that, however, the

biophysical determination of water potential gradients is essential to understand the performance of plants in their environment. In addition to the water potential of solutions (equation 7) we need the water potential in the gas phase:

$$\Psi_{\mathrm{gas-phase}} = \frac{\mu_{\mathrm{gas-phase}} - \mu_{\mathrm{saturation}}}{V_{H2O}} \tag{8}$$

where $\mu_{\mathrm{gas-phase}}$ is the chemical activity of water in the atmosphere and $\mu_{\mathrm{saturation}}$ the chemical activity at temperature dependent saturation. Physical meteorological tables provide values of the latter. The former is obtained from measurements of relative humidity in the atmosphere which are easy to perform (RH):

$$\mathrm{RH} = \frac{\mu_{\mathrm{gas-phase}}}{\mu_{\mathrm{saturation}}} \cdot 100\ (\%) \tag{9}$$

In this way, water potential gradients can be assessed between solutions and the atmosphere, i.e. between tissues and organs within the plants and the environment. The $\Delta\Psi$ between the soil and leaves may be in the range of 1 MPa, while the $\Delta\Psi$ between leaves and the atmosphere can be 100 MPa [76]. Thus, there is an enormous driving force for water transport between the plants and the atmosphere, for the so called transpiration. It energizes the flow of water, i.e. the transpiration stream, in the conduits of the xylem. Adhesion of the dipole molecules of H_2O to the cell walls of the conducting elements and cohesion of the H_2O molecules between each other allow water columns to ascend under the tension of $\Delta\Psi$. This cohesion-tension theory appears to be the dominant mechanism quantitatively [43,98,106] although it is questioned by some authors [65,109]. Under some conditions osmotic processes along the path of the transpiration stream may also participate, but this is still debated [95,100]. A strong demand of further biophysical research is given to understand these principally and ecologically important problems.

Osmotic processes of root pressure and along the conduits of long distance transport in roots, stems and leaf-petioles also participate in refilling of conduits, i.e. in repair, after cavitation and embolism have occurred under stress of drought [15,21] and frost [63]. Structure-function relations governing the distribution of water in the whole plant along the soil-leaf continuum are the biophysical parameters of hydraulic architecture of plants [95], namely

- specific stem conductivity K_s Ξ [kg s^{-1} m^{-1} MPa^{-1}],
- cross section area per unit leaf area (Huber value) H_v [dimension less],
- conductive stem per unit leaf area K_l Ξ [kg s^{-1} m^{-1} MPa^{-1}].

They are characterizing the environmental responses of plants and the eco-physiological regulation of plant water status.

3. Photobiology

Light is electro-magnetic radiation. This is a domain of physics. Thus, evidently photobiology is biophysics. The two main fields of plant photobiology are photosynthesis and related processes and developmental biology. Photobiology of photosynthesis deals with quantitative aspects of radiation, i.e. the intensity of photosynthetically active photon flux density (PPFD) in the wavelength range of 400 to 750 nm. Photobiology of plant development deals with qualitative aspects of the regulatory

effects of light of different wavelengths. Both are extraordinarily extensive areas of research and knowledge. It is far beyond the scope of the present assay to go to any details. However, in the endeavor of advocating perspectives of biophysics in plant biology we may not overlook them and need to allude to some general principles.

3.1. Photosynthesis

In photosynthesis chlorophyll molecules absorb photons, and electrons in the pigment molecules get excited to a first, second and third singlet state. The latter two relax by the emission of heat. From the first singlet excited state the electrons flow via a chain of biochemical redox systems in the thylakoid membranes towards pyridine nucleotides (nicotineadenine-amide-dinucleotide, NAD, in some photosynthetically active bacteria, and nicotineadenine-amide-dinucleotide-phosphate, NADP, in other bacteria, cyanobacteria and eukaryotes) as acceptors. The reduced NAD(P)H drives the photochemical work of the reduction of CO_2 in assimilation and O_2 in photorespiration with the respective carboxylase and oxygenase functions of ribulose-bis-phosphate carboxylase/oxigenase (RubisCO) as well as reduction of NO_3^- and SO_4^{2-}. From the first excited singlet state electrons can also reach a triplet state from where energy transfer leads to the formation of reactive oxygen species (ROS) and the destructive photochemical work of photodamage.

From the first singlet excited state the chlorophyll can relax directly to the ground state by the emission of red light shifted to the far red region of the spectrum as compared to the 680 nm maximum of the excitation of chlorophyll *a* in photosystem II (PSII). This is called chlorophyll fluorescence. The intensity is weak. However, it can be readily measured by physical photometric techniques. It is an alternative to other pathways of energy dissipation and chlorophyll relaxation. As such it is a direct record of the condition and operation of PSII. Biophysical measurements of chlorophyll fluorescence have proven to be of enormous value in a huge range of studies in plant physiology and the physiological ecology of photosynthesis. Two equations are standing out, which may be given here to characterize the most important features of PSII in the dark adapted and light adapted states, respectively, of green photosynthetic cells and leaves:

$$\text{Potential quantum yield of PSII of dark adapted samples} = (F_m - F_0)/F_m \tag{10}$$

$$\text{effective quantum yield of PSII of light adapted samples} = (F'_m - F)/F'_m \tag{11}$$

where F_m and F'_m are the maximum fluorescence yields of the dark and light adapted samples, respectively, and F_0 and F_m give the respective ground fluorescence in weak measuring light [7,25,40,62,84]. From equation (11) the photosynthetic electron transport rate (ETR) can be derived as a quantitative measure:

$$ETR = 0.86 \times 0.5 \times [(F'_m - F)/F_m) \times PPFD] \tag{12}$$

where the factor 0.86 indicates that on average 14 % of the incident radiation are reflected and the factor 0.5 accounts for distribution of radiation between the two photosystems, i.e. PSI in parallel to PSII.

Evidently studies of the primary reactions of light absorption and electron transport in photosynthesis are intrinsically biophysics. We should note in passing that design and construction of fluorometer equipment used requires a lot of physics. This also applies to porometers used for

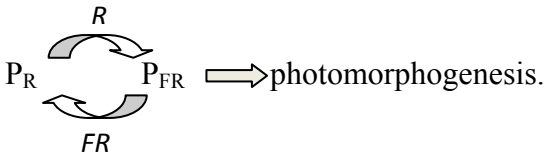
studying gas-exchange, i.e. of CO_2 and water vapor, in photosynthesis. This cannot be covered in the present assay but is of eminent importance for the links between plant biology and physics.

However, what we must remember is that the photosynthetic pigments and the redox molecules serving photosynthetic electron transport are imbedded in and interacting with proteins in the thylakoid membranes. A large number of different specific protein molecules are involved so that thylakoid proteomics is an important issue [24,107]. Once again in our survey, we encounter the need of close cooperation of biophysics and molecular biology.

3.2. Signaling and development

As an example of light signaling I have already mentioned vision above. More generally light signaling for regulation of development is a vast field in plant biology. (In the brief survey here I follow Schopfer and Brennicke [83]). The biophysical steps are the absorption of light by various pigments. These are sensor pigments present only in low concentrations as compared to the mass pigments, such as the chlorophylls and other pigments in photosynthesis. The pigments are bound as chromophores to proteins forming the chromo-proteins effective in light signaling. There are two types of photosensors for near-ultraviolet and blue light in the wavelength-region of 340–520 nm, namely cryptochromes and phototropins, and phytochrome in red light, each with families of proteins. For example for phytochrome we can distinguish 5 forms of phytochrome A to E. The chromophores are pterine and a flavineadenine-dinucleotide in chryptochromes, a flavine-mononucleotide in phototropins and an open linear tetrapyrrole in phytochromes.

The activation of phytochromes occurs in a biophysical equilibrium of red light (R at 660 nm) and far-red light (FR at 730 nm). There is a red absorbing form, P_R, which after the absorption of R changes its conformation by a shift of the position of one of the terminal pyrrole rings and thus is transformed into the far-red absorbing form, P_{FR}. The latter returns to the P_R form after the absorption of FR:



Under these light qualities the conversions between P_R and P_{FR} are strictly reversible. Quantitative radiation biophysics are involved in assessing phytochrome reactions at various light intensities as we distinguish three types, namely (i) very low fluence reactions, (ii) low fluence reactions and (iii) high irradiance reactions.

The primary reactions of light absorption constitute the biophysical input pathways of signaling. Then there is a direct link to molecular biology. The pigments directly interact with the protein moieties of the chromo-proteins. For instance in phytochrome the change of the conformtion of the tetrapyrrole chain after absorption of R effects a conformation change of the phytochrome protein. This elicits a phosphorylation of the protein and its activation to the active P_{FR} form, which has the function of a kinase. In interaction with phosphorylases it can drive cascades of protein-phopshorylations in the cytosol. P_{FR} can also enter the nuclei to interact with regulators of gene activity. In this way downstream of the primary biophysical reaction of light absorption at the

molecular level in cascades and in networks a plethora of events can be regulated which we summarize under the term of "photomorphogenesis".

Cryptochromes function in blue light responses of photomorphology and chronobiology. Phototropins are active in plant movements, e.g. phototropisms, and in the blue light regulation of stomatal aperture. The range of phytochrome regulated photomorphogeneses is etraordinarily broad. There are biophysical photo-electric membrane effects. Other examples are regulation of the synthesis of enzyme proteins, such as phenylalanine ammonia lyase for anthocyanine synthesis, nitrate and nitrite reductases, chalcone synthase and lipoxigenase. Further phytochrome-photomorphogeneses comprise differentiation of plastids, regulation of seed germination, etiolement/de-etiolement, nyctinastic movements of leaflets, photoperiodism of short day and long day plants.

Of enormous ecophysiological importance is the effect of shade under vegetation. With an absorption maximum of chlorophyll *a* in PSII at 680 nm photosynthesis filters out much of the red light necessary to stabilize the active P_{FR} form. In full sun light the R/FR-ratio is 1.2, but on a forest floor it may be reduced down to 0.5. The absorption spectra of P_R and P_{FR} are overlapping and it depends of the actual wavelength given how much P_{FR} is present for photomorphogeneses. There is a photo-equilibrium at any wavelength λ, i.e.

$$(\text{Photo-equilibrium})_\lambda = P_{FR\text{-}\lambda}/P_{\Sigma(PR + PFR)} \quad (13)$$

4. The Biological Clock

The biological clock reveals itself by many endogenous rhythms in plants. These rhythms are circadian with period lengths close to 24 h when running free under constant environmental conditions [50]. Among several external control parameters of the clock we recognize blue and red light with input pathways via cryptochrome and phytochrome as receptors or "zeitnehmer". These are biophysical reactions (see above). However, current clock research is essentially molecular biology mostly using *Arabidopsis thaliana*. Among the master genes of the clock there are the morning genes *CIRCADIAN CLOCK ASSOCIATED (CCA1)* and *LATE ELONGATED HYPOCOTYL (LHY)* and the evening genes *TIMING OF CHLOROPHYLL a/b BINDING (TOC1*) in the evening. Down-stream there are additional morning and evening elements functioning as transcription factors [28,38,64,70] and a vast number of clock controlled genes (CCGs) putting a plethora of plant functions under the regime of the clock [23].

An important circadian rhythm among plants is the metabolic rhythm of crassulacean acid metabolism (CAM), and here we have identified a biophysical oscillator operating in close interdependence with the molecular oscillators. CAM is an adaptation to problems of water supply because CO_2 is fixed by phospho*enol*pyruvate carboxylase (PEPC) in the dark period, when evaporative water demand is low, leading to the formation of malic acid which is stored in the central cell vacuole over night. The malic acid is remobilized during the light period, decarboxylated behind closed stomata producing CO_2 for photosynthetic assimilation. The endogenous biological clock is behind it, since the cycle is also running under constant environmental conditions in continuous light or darkness.

PEPC is phosphorylated in its active state and the PEPC-kinase is under circadian control [17,29]. Therefore, for some while it was assumed that the endogenous CAM-rhythm is specifically a molecular process under the genetic control of PEPC-kinase expression. By contrast,

we had proposed a biophysical oscillator. It is based on a tension/relaxation mechanism of the tonoplast membrane given by the osmotic consequences of bulk vacuolar accumulation and remobilization, respectively, of malic acid. The biophysical hysteresis switch or beat oscillator is a switch between states of order of the tonoplast with (i) low order/high fluidity/high permeability for malic acid at high malic acid filling state and higher turgor pressure facilitating malic acid remobilization, and (ii) high order/low fluidity/low permeability for malic acid at malic acid empty state and lower turgor pressure facilitating malic acid accumulation, respectively [48,55]. Thus, the tonoplast is functioning as a biophysical master switch in the circadian rhythm of CAM [49].

Later studies showed that the molecular oscillator of the genetic control of PEPC-kinase and the biophysical oscillator of tonoplast state of membrane order are coupled. The gene expression of PEPC-kinase was found to be under the metabolic control of malate in the cytoplasm [10,74]. This constitutes a direct link to the biophysical malic acid influx/efflux oscillator affecting cytoplasmic malate concentrations. The endogenous rhythm of CAM is an excellent example of the integration of both biophysical and molecular mechanisms in complex physiological functions.

5. Whole Plant Integration: Self-organization and Emergence

The building blocks of plants are the organs roots, shoots and leaves. Building blocks of the organs are various tissues. We may consider these as modules. The higher organized systems, such as the organs or the entire plants are more than the sum of their parts. There is integration with self-organization from the parts. Plants are integral organisms. In these individuals properties and capacities are expressed which go far beyond the modularity of the parts. This result of self-organization is also named emergence [51]. The unitary integration of modules in the integral units of plants requires perception and transduction of signals and communication, i.e. sharing of information [52,87]. Basically there are three types of signals, chemical signals, electrical signals, hydraulic signals. The latter two are biophysical. Understanding whole plant integration is an eminently biophysical task.

Chemical signals are the primary messengers of the classic groups of phytohormones and secondary messengers, where currently increasing numbers of regulatory compounds are identified as the understanding of the complexity of networks of physiological and biochemical functions and their integration advances.

Electrical signals are action potentials (AP), variation or slow wave potentials (VP) and system potentials (SP) with rates of propagation ranging from just below 1 mm s^{-1} to 50 mm s^{-1} or even higher [52]. The systemic function of long distance electrical signaling in plants requires their transmission which occurs mainly in the long distance transport bundles and particularly in the phloem [26,27,52,86].

Hydraulic signals are pressure changes and hydraulic waves [26,59,97] with the movement of water columns in the xylem [18]. In the cells membranes, particularly the plasma membrane and the tonoplast, are the sites of sensing the turgor pressure (P) which is determined by gradients of water potential (Ψ) and osmotic potential (π) (equation 6). Ψ cannot be perceived by the cells directly but indirectly via P and π. There are three ways in which in shoots and leaves hydraulic signals of the roots can be decoded: (i) via osmosensing π, (ii) via sensing P, and (iii) by sensing mechanical forces at the cell wall or even in membranes via mechano-sensitive stretch responsive ion channels and ion pumps [52,87,91,92].

In signal transduction chains and networks the three types of signals, the chemical and the two biophysical ones can be translated into each other, there is a cross talk between physical and chemical signals. A couple of examples may show *"connections between physical, (bio)chemical and molecular signals in the plant"* [87].

Gravitropic orientation of the plant is a developmental process governed by electric and chemical signaling. Gravitropic root bending is elicited by sensing the position of the root in relation to the field of gravity in the root caps within special cells, the statocytes, by starch grains lying on a cushion of endoplasmic reticulum and functioning as statoliths. An early event observed in gravitropic reactions of roots is the so-called geo-electric effect first described by Brauner and Bünning [12] and later confirmed in detailed studies by Behrens et al. [6] and Stenz and Weisenseel [93,94], where the membrane potentials in root cap cells at the physically upper face of a root coming into a horizontal position in relation to gravity become more negative (–131 mV) than at the lower face (–93 mV) [6]. The sensing is in the tip of the root but the bending occurs by asymmetric growth further up in the elongation zone. This requires signaling which involves asymmetric distribution of the phytohormone indole-acetic acid (IAA) coming into the root from above and being transported back and of a second phytohormone cytokinine produced in the root tip [2].

An important mechanism in whole plant integration is root-shoot signaling of water relations where the roots can inform about water problems for early stomatal regulation in the leaves even if these have not yet suffered from reduced supply. Hydraulic and chemical signals can translate into electrical signals. Hydraulic surges and changes of turgor pressure are translated into electrical signals of APs, VPs and SPs [20,52]. Hydraulic signals interact with chemical signaling via the phytohormone abscisic acid (ABA) and another phytohormone interacting with root-shoot electrical signaling is cytokinin [86].

6. Bionics

Bionics links biology with technology. The term biomimetics is also used, but bionics is much more than simple mimetic imitation or copying of nature. Werner Nachtigall is distinguishing the two steps of technical biology and bionics [67]. The first one is the step from recognition of structure-function relations in the living world by the biologist with extraction of general principles leading to modeling by analog abstraction from the original biological data. It is a field in its own right but not yet bionics. Bionics then is the step from abstraction towards appropriate technical realization by the constructing engineer. There is self-acceleration in the process. Biological recognition leading to technical applications and new technical capabilities via bionics feeds back via technical biology to biological understanding. Engineering intrinsically uses physical approaches.

Structural and functional biophysics of plants giving ideas for technical developments include construction of wheels, features of wings, building principles of the hollow stems of grasses, statics of buttress roots, and effects of surfaces [8,13,56,68,89].

The wheel has probably originated from cut sections of the stems of trees. Recently super-light wheels have been developed after the three-dimensional structure of spherical diatoms. One-wing flying machines have been designed after the giant up to 15 cm wide wind dispersed winged seeds of the Cucurbitaceae *Zanonia macrocarpa*. The technical grass shoot has been constructed after the tall grass *Arundo donax* as well as the horsetail *Equisetum*, and his light building-principle combined

with mechanical strength has found many technical applications as tubes for special purposes and in constructions of many areas of engineering [89,90,102]. Buttress roots stabilize large trees and this construction principle has been analyzed for the application in reducing the notch stress in buildings and other technical constructions [58]. Studies of the sculpturing of surfaces of plants, especially of some leaves, have led to bionic surface technology [3,4,5]. Due to being covered with epicuticular wax crystals of about 110 nm diameter surfaces of leaves become super-hydrophobic, i.e. anti-adhesive for droplets of water. They are self-cleaning because the water droplets running off take mud and dirt particles along with them. The most widely known example is the leaves of the lotus plant *Nelumbo nucifera* which is a holy plant and symbol of purity in Buddhism and Hinduism. It has led to the bionic development of surface paints for self-cleaning facades of buildings, for automobiles and other surfaces technically known under the trademark Lotus-Effect^{R}. Another example of super-hydrophobic plant surfaces are the leaves of the floating water fern *Salvinia*. The highly complicated structured surfaces have super-hydrophobic elastic hairs between which an air layer is kept under water. Ships covered with such air-retaining surfaces would benefit from the Salvinia-Effect by significantly reduced friction when gliding through water which would help to reduce fuel consumption.

On the molecular level it is attempted to realize the dream of using the principle of photosynthetic electron transport in thylakoid membranes for technical provision of energy by a biomimetic kind of photo-voltaics. In the so-called Graetzel-cell two glass electrodes, anode and cathode, are used, where the anode contains pigment molecules triggering a light-driven flow of electrons, i.e. current [77]. Initially these were actually chlorophyll molecules; later they were replaced by synthetic pigments. It is a promising biophysical approach deserving more study and technical development [105].

7. Life in Space: an Extreme Biophysical Perspective

Exploration of space is the realm of physics, astrophysics. Speculations about life elsewhere in the universe are nourished by the statistical argument that the increasing discovery of huge numbers of earth-like planets in the universe makes it likely that life exists elsewhere although distances are so immense that we shall never have a chance to learn about that. The question is immensely biophysical: first, because of the astrophysical description of space; second, as life elsewhere would also need primary producers feeding it and all the physical processes of energy transduction involved, and would be subject to thermodynamics of dynamic steady state “equilibria”.

8. Outlook: Quo Vadis Plant-biophysics in the Molecular Age?

This brief list of some hot-spots of biophysics in plant biology and tour of the horizon shows that biophysics is indispensible for putting together the puzzles of modules obtained in trans-sub-disciplines of plant biology to understand emergence of higher integrated systems. It is necessary for comprehending plant life and assessing the important services plants make to the function of other living systems, such as biomes and ecosystems including agro- and forest ecosystems, sustaining the existence also of man on our globe. Biophysics is the basis of many innovations, such as bio-mimetic technologies. Plant-biophysics is not fading away in the molecular age. By the very contrary, all the hot spots show that it must merge with molecular biology to really foster progress of basic

understanding and sustainable management of plant life (Figure 1). In quite a number of cases as shown in this article such union is already effective and proves to have considerable potential for managing the future. Sustained and increased practice of biophysics with teaching and research deserves strong encouragement.

Conflict of Interest

The authors declare no conflict of interests.

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