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Application of Fixed-Length Ultrasonic Interferometry to Determine the Kinetics of Light-/Heat-Induced Damage to Biological Membranes and Protein Complexes

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Abstract: This manuscript describes the application of a fixed-length ultrasonic spectrometer to determine the kinetics of heat- and photo-induced damage to biological membranes and protein complexes and provides examples of the test measurements. We implemented a measurement scheme using the digital analysis of harmonic signals. To conduct the research, the fixed-length ultrasonic spectrometer was modernized: the speed was increased; lighting was supplied to the sample cells; the possibility of changing the gas atmosphere and mixing the sample was given. Using solutions containing natural concentrations of deuterium oxide, a high sensitivity of the spectrometer was shown. The spectrometer performed well in the measurement of phase state of dimyristoylphosphatidylcholine liposomes, both in the absence and in the presence of additions, which are capable of changing the lipid properties (sodium dodecyl sulfate, palmitic acid, and calcium ions). The heat- and photo-induced changes in the state of photosystem II core complexes were demonstrated using a fixed-length ultrasonic spectrometer. Transitions at 35.5 °C, 43.5 °C, 56.5 °C, and 66.7 °C were revealed. It is proposed that the transitions reflect the disassembly of the complexes and protein denaturation. Thus, the present study demonstrates that a fixed-length ultrasonic spectrometer can be applied to determine the kinetics of heat- and photo-induced damage to biological membranes and protein complexes.

Keywords: ultrasound; fixed-length ultrasonic spectrometer; biological membranes; protein complexes; phase states; phase transitions; photosystem II



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1. Introduction

Biological membranes are unique heterogeneous three-dimensional structures, consisting of proteins embedded in a lipid matrix, the architecture and stability of which are strongly related to their functions. One of the most important characteristics of biological membranes is their phase state, including the state of protein molecules integrated into the membrane. It is known that lipids, the main structural components of biological membranes, may have three phase states: liquid (liquid crystal), ripple and solid (gel) [1]. The phase transition point depends on many factors: the structure and packing of lipid molecules, hydrostatic pressure, salt concentration, and so on. Phase transitions of lipid membranes are the basis of important biological processes: the fusion of cells, osmosis, the transport of substances, etc. [2–7]. Proteins can also exist in several thermodynamically stable states, for example the native state, denatured state, and compact state of the molten globule with a loss of unique side chain packaging. The transitions between these states in

proteins also resemble the phase ones: with a smooth change in conditions (temperature, pH, or ionic strength), there are drastic changes in the structure of the protein molecule, the cause of which is also a large amount of weak interactions that determine the tertiary structure [8–13].

Despite the fact that the structure of the lipid bilayer is quite stable, individual phospholipid molecules have some freedom of motion. The structure and stability of the lipid bilayer depend on the lipid composition and change with temperature. Lipids form a semi-solid phase (gel, liquid crystal state) in the bilayer at temperatures below physiological. In this state, all motions of individual lipid molecules are strictly limited, phospholipids are arranged in a strictly ordered manner, and the hydrophobic hydrocarbon tails of phospholipid molecules are completely extended parallel to each other. As a result, the surface area of the membrane is greatly reduced. At temperatures above physiological, the hydrocarbon chains of fatty acids are in constant motion, due to the rotation of the hydrocarbon bonds of long acyl chains. In this liquid-crystalline (or liquid-disordered) state, the inner region of the bilayer resembles a sea of lipids in constant motion. In the liquid state, structural transitions are possible due to thermal motion: the molecules are bent; their parallelism is broken in some places. As a result, the surface area of the membrane significantly increases. At intermediate temperatures, biomembrane components, including proteins, exist in a liquid-ordered state [14]: the thermal motion is reduced in the acyl chains of the lipid bilayer, but lateral movement still occurs. These differences in the state of the bilayer are easily observed in monolipid liposomes. For example, the solid-to-liquid phase transition of the synthetic phospholipid dipalmitoylphosphatidyl choline occurs over a very narrow temperature range, down to as little as one degree Celsius (43 °C). At the same time, biological membranes, which contain many lipids with a variety of acyl chains, do not show fast phase transitions with temperature changes.

The thermal phase transition is the most-studied phase transition. At the same time, a number of compounds can affect the packing and ordering of phospholipids in a lipid bilayer membrane: local anesthetics, short alcohols, and hydrocarbons make the membrane more liquid (including the decrease in the phase transition temperature); at the same time, calcium ions, long alcohols, and hydrocarbons structure the membrane (including the increase in the phase transition temperature) [15,16]. In this case, it is correct to say that a chemotropic phase transition occurs. One of the important membrane modifiers is cholesterol [17,18]. Cholesterol molecules are located between phospholipid molecules. They order the bilayer in the liquid state and disorder it in the solid state, thus reducing the differences between the liquid crystal and solid crystal structures.

Currently, the main approaches for studying the state and phase transitions are differential scanning calorimetry, NMR spectroscopy, Fourier transform-infrared spectroscopy, quartz crystal microbalance with dissipation monitoring, etc. [19–33]. However, most of these approaches require expensive equipment and expendable materials. It is a good alternative to assess the state and phase transitions of membranes and protein molecules using an ultrasonic spectrometer [34–38]. The method of ultrasonic differential spectrometry combines high sensitivity, accuracy, and a small volume of the test sample [39]. The principle of measurement is to study the resonant properties of a composite resonator that includes a sample [40,41]. The method allows investigating the properties of solutions, colloids, suspensions, and emulsions. Measurement of the speed of sound in a sample allows one to automatically measure the compressibility of samples. The measurement of the compressibility of suspensions and emulsions is an approach that allows estimating the amount of water in the hydration shell of hydrophobic particles or the change in the number and state of these particles. In addition, the method makes it possible to measure acoustic impedance, excess enthalpy, miscibility, and the compatibility of mixtures. Compressibility, the derivative of volume with respect to pressure, is a physical parameter that characterizes the potentials of intermolecular interactions in a condensed medium and the response of the molecular structure to pressure. These two factors determine the instantaneous (high-frequency) and relaxation parts of the compressibility, respectively. The value of the

compressibility determines the phase velocity of sound in the medium, and its relaxation part determines the value of sound absorption. Therefore, simultaneous measurements of the velocity and absorption of ultrasound, as well as the density of the medium make it possible to determine the compressibility of the medium and its relaxation part with very high accuracy. In addition, the compressibility of an aqueous solution is highly dependent on the hydration of the solute. Almost all molecular processes (including phase transition) in solution are accompanied by a change in hydration, which can be recorded and characterized by the measurement of compressibility [42]. On the one hand, compressibility is a sensitive characteristic of the state of aggregation of a substance. On the other hand, a protein molecule and a lipid membrane are systems with a large number of internal degrees of freedom, being delimited from the medium. Therefore, they can be considered as a phase of small size, and their properties can be described in “macroscopic” terms [43].

One of the most accurate methods is the assessment of the state and phase transitions of biomolecules by changing the compressibility and absorption of ultrasound using a setup containing a fixed-length ultrasonic. To do this, we previously used a phase-locked loop (PLL) [41], which was later replaced by an alternative measurement scheme based on the methods of digital analysis of harmonic signals [44–46]. In the present work, the installation was further modernized, which made it possible to increase the speed, as well as to perform studies to determine the kinetics of heat- and photo-induced damage to biological membranes and protein complexes. The approach proposed in this work can be the basis for obtaining such important characteristics of a phase transition as the type of melting and denaturation of proteins, interfacial tension, and the rate constant of the elementary act of the growth and decomposition of a new phase, providing information about the physical properties of lipid membranes and protein molecules and on intermolecular interactions in aqueous solutions. This method combines high sensitivity, accuracy ($10^{-4}\%$), and a small volume (100 μL –1000 μL) of the test sample [39].

2. Materials and Methods

The dependence of the relative velocity of ultrasound in water on temperature at various concentrations of deuterium oxide were analyzed using different ratios of light water (“Legkaja voda, Standart”, Komponent-Reaktiv, Moscow, Russia) (without deuterium oxide) and ordinary water. The ordinary water was obtained by distillation and subsequent deionization of tap water.

Dimyristoylphosphatidylcholine liposomes were prepared by fifteen times of extrusion through a 200 nm membrane at room temperature [45]. Prior to this, the dimyristoylphosphatidylcholine (Sigma Aldrich, Merck Group, Darmstadt, Germany) was hydrated for several hours, followed by five freezing/thawing cycles. This procedure led to the formation of unilamellar vesicles [45,47–49]. Ultrasonic measurements were performed in the medium containing 50 mM KCl (Sigma Aldrich, Merck Group, Darmstadt, Germany), 20 μM EGTA (Sigma Aldrich, Merck Group, Darmstadt, Germany), 10 mM Tris-HCl (pH 8.5) (Sigma Aldrich, Merck Group, Darmstadt, Germany), and 1% ethanol (Chimmed Group, Moscow, Russia) (added with palmitic acid (Sigma Aldrich, Merck Group, Darmstadt, Germany)). The concentration of lipids used in the measurements was 1 mg/mL.

The isolation of photosystem II (PSII) core complexes was performed according to [50] with some modification [51]. The PSII membrane preparations used for the isolation of PSII core complexes were obtained in accordance with [52]. Removal of the water-oxidizing complex (WOC) from PSII core complexes was performed by 1 h of incubation in the presence of 5 mM NH_2OH (Sigma Aldrich, Merck Group, Darmstadt, Germany) and subsequent purification on a Q-Sepharose column (GE Healthcare Bio Sciences, Uppsala, Sweden) [51]. Photoinhibition of “native” PSII core complexes was achieved by continuous illumination ($\lambda = 400\text{--}800\text{ nm}$, 1500 $\mu\text{mol photon s}^{-1}\text{ m}^{-2}$) provided by LED (JH-5WBVG14G24-Y6C, Ledguhon, Guangzhou Juhong Optoelectronics Co., Ltd., Guangzhou, Guangdong, China) in a medium containing 50 mM MES (pH 6.5) (PanReac AppliChem, ITW Reagents, Glenview, IL, USA), 35 mM NaCl (PanReac AppliChem, ITW Reagents,

Glenview, IL, USA), and 0.003% (w/v) n-dodecyl- β -D-maltoside (PanReac AppliChem, ITW Reagents, Glenview, IL, USA) at a chlorophyll concentration of 18 $\mu\text{g/mL}$. The photoinhibition procedure was performed in the ultrasonic spectrometer cell. The ultrasound study of heat- and photo-induced damage to photosystem II core complexes was performed in the presence of 50 mM MES (pH 6.5), 35 mM NaCl, and 0.003% (w/v) n-dodecyl- β -D-maltoside at a chlorophyll concentration of 18 $\mu\text{g/mL}$ (for heat-induced processes) or 7 $\mu\text{g/mL}$ (for light-induced processes).

The Chl concentration was determined in 80% acetone (Chimmed Group, Moscow, Russia) [53]. The manganese content in the PSII preparations was controlled using the atomic absorption spectrometer KVANT-2A (Cortec, Moscow, Russia).

During the ultrasonic measurement, the samples in both the reference and experimental cells were mixed with a magnetic stirrer. All measurements were repeated at least three times.

3. Results

3.1. Setup

Figure 1 shows the structure of the measuring scheme of a fixed-length digital computer ultrasonic spectrometer. In comparison with the previous version [44], in order to overcome the limitations of the method associated with the speed of measuring the phase-frequency characteristic, the measurement channel of the experimental cell was upgraded. The directions of modernization were as follows:

1. Acceleration of the operation of the controller that tunes the DDS generator: an 8-bit ATmega family (Atmel, Microchip Technology Inc., Chandler, AZ, USA) with a clock frequency of 16 MHz was replaced with a 32-bit SAM3 controller (Atmel, Microchip Technology Inc., Chandler, AZ, USA), that is replacing the Arduino Uno controller with the Arduino DUE controller.
2. Transition from a circuit with one oscillator and one oscilloscope serving two channels and a switch to a circuit with one 125 MHz master oscillator and two DDS generators.
3. Replacement of the digital oscilloscope in the channel of the experimental cell with a high-speed recorder with 32 MB of RAM and a USB3.0 interface. The recorder is started by an external trigger. The trigger signal is generated by the Arduino DUE.
4. The measurement procedure in the channel of the experimental cell was changed. The Arduino DUE controller independently forms a grid of 15 frequencies and the 16th interval with the generator turned off. The entire frequency grid is automatically fed to the input of the experimental cell without the participation of a personal computer. The measurement result (in the form of a continuous recording with a sampling frequency of 50 MHz and eight million samples long) is transferred to a computer and analyzed. Thus, the procedure for measuring the characteristics of one resonant peak takes 160 ms, rather than 4 s as in the previous version.

In addition, the measuring cells were additionally equipped with magnetic stirrers (2 in Figure 2), LEDs (JH-5WBVG14G24-Y6C Ledguhon, Guangzhou Juhong Optoelectronics Co., Ltd., Guangzhou, Guangdong, China), and capillaries for supplying or evacuating gases mounted in the covers (5 in Figure 2).

The program used to implement the algorithm for signal analysis (using digital signal processing) and control of the experimental conditions was developed with C++. The program performs the following tasks in real-time: (i) obtains raw digitized sinusoidal signals at the input and output of each cell, (ii) sets the frequency of the DDS generator, (iii) calculates the phase frequency dependence and amplitude transmission coefficient (AFC) and determines the position of the maximum of the resonant peak in the frequency range, (iv) visualizes the data, and (v) scans the temperature in the cells and controls the thermostat.

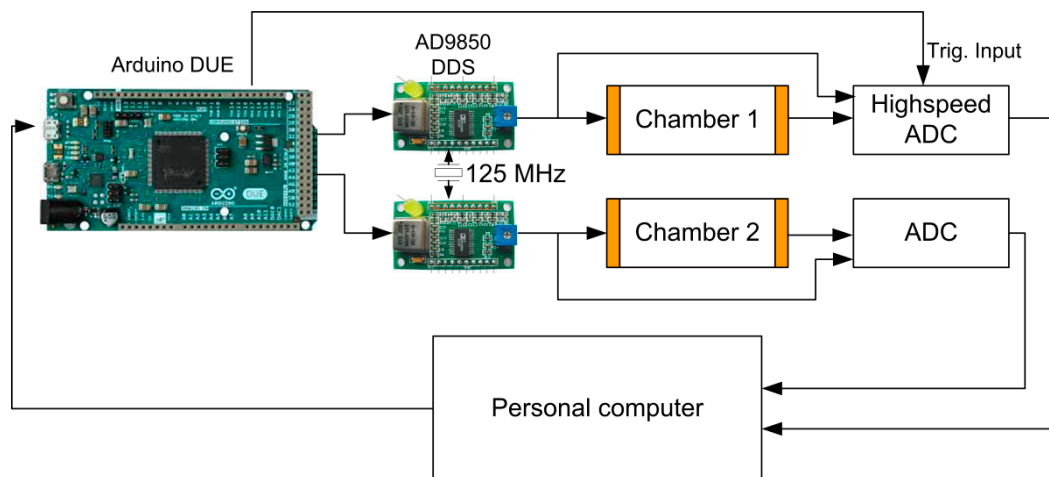


Figure 1. Block diagram of the setup containing a fixed-length ultrasonic spectrometer.

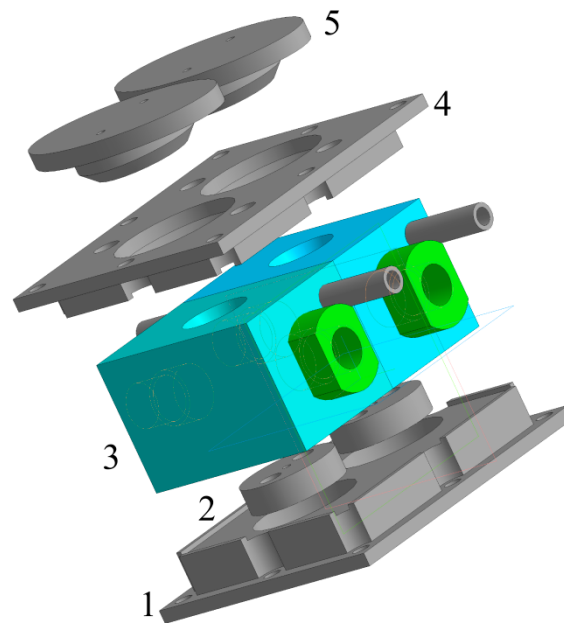


Figure 2. The 3D layout of the fixed-length ultrasonic spectrometer. (1) Bottom plate (PETG, 3D-printed); (2) magnet stirrer rotor (PETG, 3D-printed); (3) ultrasound measuring chambers (nickel-plated brass, machining); (4) top plate (PETG, 3D-printed); (5) caps (PETG, 3D-printed). DC brush motors, magnets 6×3 , channels for supply/removal of gases to cells, and LEDs are not shown.

In comparison to the method described earlier [44], in the present work, the principle of determining the position of the resonance peak was changed (Figure 3). If the algorithm did not change much for the sample cell, then a simplified procedure was used for the reference cell. The phase frequency dependence near the resonant frequency was measured to perform steady-state measurement of the frequency and width of a resonant peak in a cell with a sample. The estimation of the position of the resonant peak was made by determining the position of the zero of the second derivative of the phase frequency dependence (Equation (1)). To obtain one point of the second derivative of the PFC, it is necessary to perform three dimensions of phase frequency dependence. This was repeated five times at small, regular frequency intervals. After that, the dependence of the second derivative of the phase frequency dependence was approximated by a straight line, which intersects the zero of the ordinate at the position of the resonant peak. In parallel, the first derivative was calculated (Equation (2)) for the calculation of the fork pitch at the

next determination of the position of the resonance peak (Equation (3)). The width of the resonance peak was calculated using Equation (4).

$$\phi''(f) = \frac{\phi(f + \Delta f_{[\text{yellow}]\phi}) + \phi(f - \Delta f_{\phi}) - 2\phi(f)}{\Delta f_{\phi}^2} \quad (1)$$

$$\phi'(f) = \frac{\phi(f + \Delta f_{\phi}) - \phi(f - \Delta f_{\phi})}{2\Delta f_{\phi}} \quad (2)$$

$$\Delta f_{\phi} = a_{\phi} \frac{\pi}{4\phi'(f)} \quad (3)$$

$$\Delta f = \frac{\pi}{2\phi'(f)} \quad (4)$$

where Δf_{ϕ} is the fork pitch and a_{ϕ} is the trimming factor. We used Δf to determine the Q-factor for the energy losses in our resonant system (Equation (5)):

$$Q = \frac{f}{\Delta f} \quad (5)$$

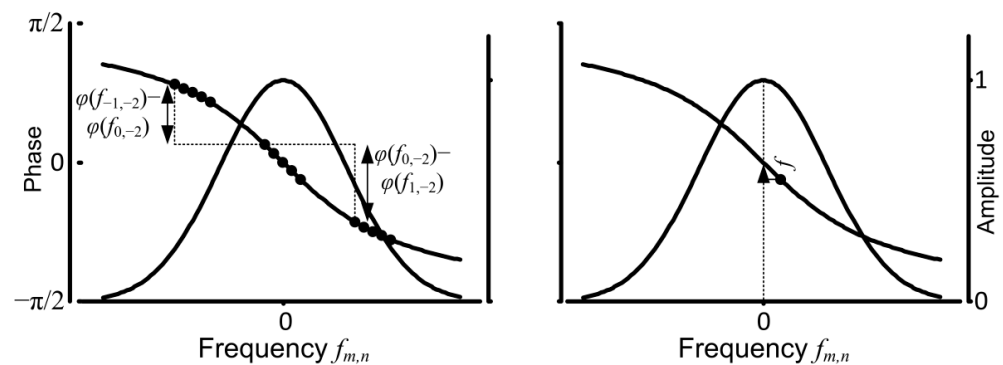


Figure 3. Comparison of the method for the determination of the position of the resonant peak in the cell with the test sample (left) and in the control cell with water (right).

As the Q-factor rises with losses falling, we used the $1/Q$ parameter to characterize the losses in the system due to scattering and viscosity.

As mentioned above, a simplified procedure was used for the reference cell, in which the phase was determined once for a frequency close to the frequency of the resonant peak. The current position of the resonant peak was determined by linear regression of the phase frequency dependence with a known slope. As a reference substance, it is better to use a known substance, for example pure water.

3.2. Measurements

Figure 4 presents the results of the measurement of the relative velocity of ultrasound in water. Line 1 indicates that the ultrasonic spectrometer had a good stability in all temperature ranges. Replacement of 50% (line 2) or 100% (line 3) light water in the experimental cell with ordinary water led to a decrease in the relative velocity of ultrasound. Moreover, this difference increased with increasing temperature. If we take into account the content of deuterium oxide in ordinary water (about 0.015%), the presented data confirm the high sensitivity of the ultrasound spectrometer.

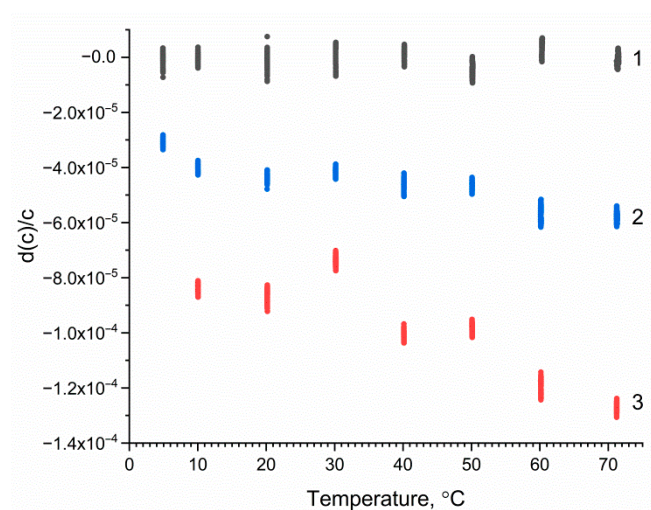


Figure 4. Dependence of the relative velocity of ultrasound in water on temperature at various concentrations of deuterium oxide. (1) Light water (without deuterium oxide), (2) 50% light water and 50% ordinary water (with a natural amount of deuterium oxide), and (3) ordinary water. There was light water in the reference cell. The ordinary water is deionized distilled tap water. c is the velocity of ultrasound in the reference cell; $d(c)$ is the difference of the velocity of ultrasound in the sample cell and in the reference cell.

Figure 5 shows the influence of the addition of sodium dodecyl sulfate (SDS), palmitic acid, and Ca^{2+} on the phase state of dimyristoylphosphatidylcholine (DMPC), a kind of phospholipid, which can be used to prepare liposomes. A sharp decrease of the ultrasound velocity at 23.85 °C reflects a change of the phase state of the liposomes. The addition of 20 μM SDS (a known agent, which destabilizes the membrane structure [54–61]) led to a slight decrease in the temperature of the phase transition (to 23.25 °C). Palmitic acid (20 μM), in contrast to the effect of SDS, increased the phase transition temperature and decreased the cooperativity of the transition (two phase transitions were observed: at 26.10 °C and 28.90 °C). These data are consistent with the previous data [62–66], including those obtained using ultrasonic measurements [67]. The addition of 200 mM Ca^{2+} eliminates the SDS- and palmitic acid-induced changes, probably due to the binding of Ca^{2+} to palmitic acid [46].

It is known that photosystem II is the pigment–protein complex, which consists of 20 protein subunits and about 20 lipid molecules [68–71]. The study of the state and phase transitions in such large complexes is a very difficult task, since the contribution of each component of the complex will overlap with the others. Figure 6A shows the kinetics of the ultrasonic velocity in the suspension containing untreated photosystem II (PSII) core complexes (black curve). The relative ultrasonic velocity gradually decreased with increasing temperature. The decrease in velocity with the temperature increase was nearly linear. However, in some parts of the kinetics, a decrease in the slope angle was observed, which may indicate changes in the protein complexes. The calculation of the derivative of the kinetics makes it possible to visualize the changes in the kinetics (Figure 6B). There are several regions of sharp growth on the derivative curve, which may indicate the decomposition of protein complexes into small components or a change in the structure of the peptides themselves. Several regions of this kind can be distinguished on the curve. The first starts at 43.5 °C, the second at 56.5 °C, and the third at 66.7 °C. Preliminary photoinhibition of the samples somewhat changed the character of the curves (Figure 6A,B, red curves). The relative ultrasonic velocity increased, which can indicate light-induced damage to the complexes (Figure 6A). The first shoulder on the derivative (observed at 43.5 °C) decreased greatly, which may indicate that those PSII components that undergo changes at this temperature were damaged by light prior to the ultrasonic measurement (Figure 6B). The curve shows a significant increase in the amplitude of the second shoulder (observed at 56.5 °C). Further, the character of both the changes in the

ultrasound velocity and their derivatives was practically the same in both types of samples (native and photoinhibited).

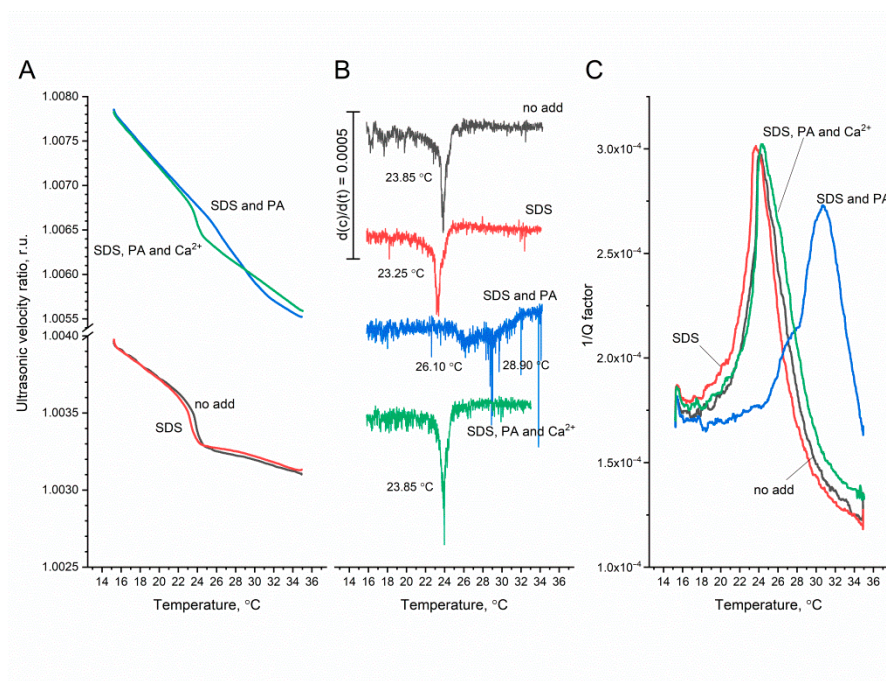


Figure 5. Effect of sodium dodecyl sulfate (SDS), palmitic acid (PA), and Ca^{2+} on the phase state of dimyristoylphosphatidylcholine liposomes. Dependence of the relative velocity of ultrasound (A), its derivative (B), and the $1/Q$ -factor (C) on temperature. Measurements were performed in the medium containing 50 mM KCl, 20 μM EGTA, 10 mM Tris-HCl (pH 8.5), and 1% ethanol (added with palmitic acid).

Figure 6C describes the dependence of the Q -factor (which can describe the change in the shape or size of particles) on temperature. The figure shows that the initial value of the Q -factor for samples pre-treated with high-intensity light was higher than for untreated samples. This may indicate that PSII core complexes were damaged as a result of pre-illumination. When the temperature reached 33.5°C , the Q -factor significantly increased in both types of samples and then did not change. At the same time, its value became the same for untreated and pre-illuminated preparations. It is important to note that the presented curves were the result of filtering the original curves (shown in the insert in Figure 6D). The original curves showed that the signal-to-noise ratio also changed with increasing temperature, which is reflected in Figure 6D. It is shown that the value of the Q -factor RMS began to increase with increasing temperature, which correlates well with the data presented in Figure 6A–C. The Q -factor RMS returned to the initial values when all the main processes, reflected in Figure 6B, ended.

It is known that photosystem II complexes depriving the water-oxidizing complex are very sensitive to photoinhibition [72–76]. Therefore, we decided to perform a study of the kinetics of photoinhibition using preparations with the WOC removed. In order to avoid additional thermal inactivation of the preparations, it was decided to conduct the experiments at a low temperature ($\approx 6.4^\circ\text{C}$). Figure 7 shows the photoinduced changes of the relative velocity of ultrasound in the suspension containing photosystem II core complexes with the removal of the WOC. Before illumination, the ultrasonic velocity remained stable.

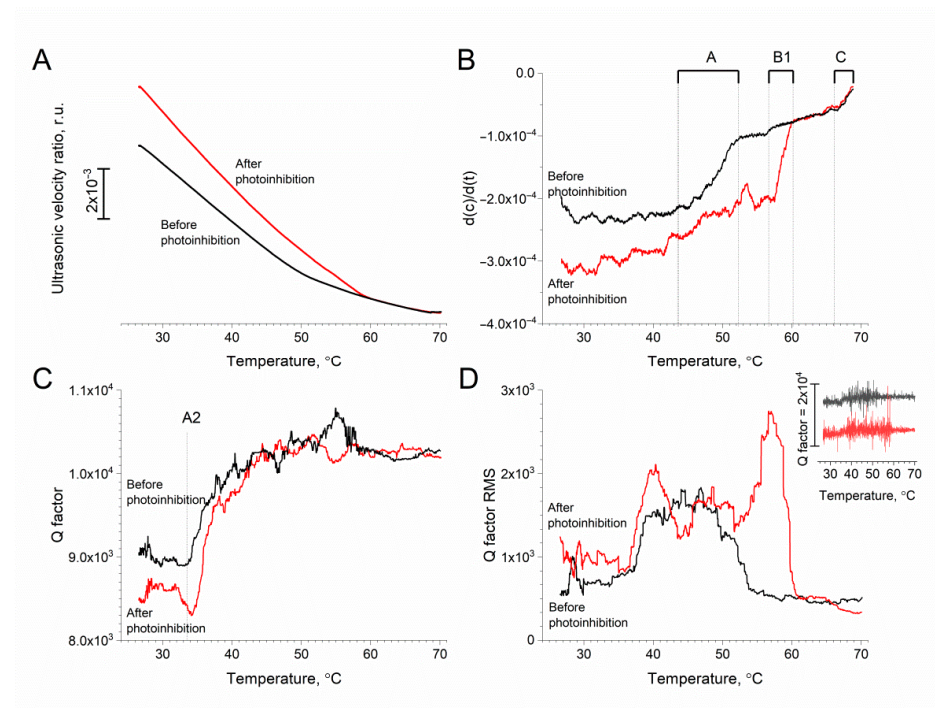


Figure 6. Ultrasound study of heat-induced damage to photosystem II core complexes before and after the photoinhibition procedure. (A) Relative velocity of ultrasound, (B) its derivative as a function of temperature, (C) the Q-factor, and (D) the Q-factor RMS as a function of temperature. Letters in (B,C) indicate transitions: A (43.5 °C), B1 (56.5 °C), C (66.7 °C) (the nomenclature accepted in [26] was used), and A2 (33.5 °C) (the nomenclature accepted in [28] was used).

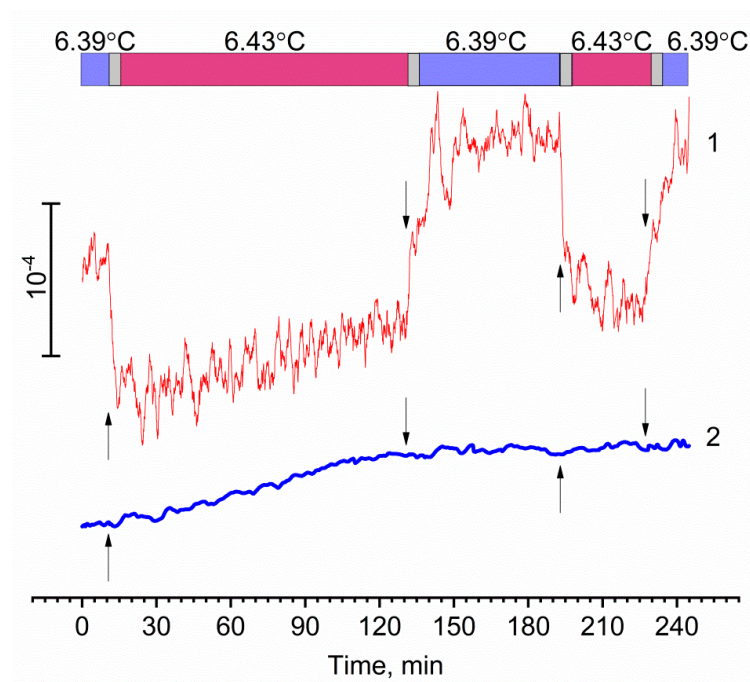


Figure 7. Photoinduced changes of the relative velocity of ultrasound in the suspension containing photosystem II core complexes with the removal of the water-oxidizing complex. The kinetics are presented before (1) and after (2) temperature compensation. The panel at the top of the figure shows the change of temperature in the cells induced by illumination. ↑ and ↓ represent the steady-state actinic light ($\lambda = 400\text{--}800\text{ nm}$, $1500\text{ }\mu\text{mol photon s}^{-1}\text{ m}^{-2}$) being on and off, respectively.

The kinetics of the photoinduced changes of the relative velocity of ultrasound can be divided into two parts: a fast phase, associated with sample heating, and a slow phase, reflecting photoinduced changes occurring in PSII complexes. The fast phase is a completely reversible decrease in the relative speed of ultrasound immediately after turning on the light, which is completely dependent on the heating of the sample ($\Delta t = 0.04\text{ }^{\circ}\text{C}$). The slow phase is a gradual irreversible increase in the speed of sound as a result of the photoinhibition of the samples, which was not detected upon repeated illumination. Note that the rate of the light-induced increase of the ultrasound velocity after the second illumination was three-times lower than after the first one. These data correlate well with those presented in Figure 6A (increase in the speed of sound in the photoinhibited sample).

Thus, it was shown that the fixed-length ultrasonic spectrometer can be used to determine the kinetics of the light-/heat-induced damage to biological membranes and protein complexes.

4. Discussion

The development of technologies for the study of the phase state of biological samples concentrates on the increase of the resolution and on the decrease of the noise in comparison to the signal. Ultrasonic technologies are also constantly being developed [77–87]. However, the majority of the attention is paid either to fairly simple or model objects or to the assessment of the quality or readiness of food products, as well as research for medical purposes [34–40,45,46,84,85,88–93]. In our work, we tested the potential possibility of studying the state and phase transitions of a large and complex multiprotein, the photosystem II of higher plants (containing peptides, lipids, photosynthetic pigments, and electron transport cofactors). Photosystem II (PSII) is the pigment–protein complex located in the thylakoid membrane of chloroplasts and cyanobacteria. PSII catalyzes the transformation of light energy into electrochemical energy with separate charges [94]. A by-product of PSII's operation is molecular oxygen. A minimal molecular structure capable of water oxidizing is PSII core complexes. X-ray analysis of PS2 core complexes isolated from cyanobacteria showed that each PSII monomer consists of 20 protein subunits, 20 lipid molecules, as well as 35 chlorophyll and 12 carotenoid molecules [68,69,95,96]. Modernization of a fixed-length ultrasonic spectrometer, the acceleration of the speed, changes in the algorithm of determining the position of the resonance peak, and mixing the sample allowed performing measurements of heat-induced damage to PSII. In addition, such measurements became possible in the direction from low to high temperature without degassing of the measuring medium. Before the modernization, such measurements were not possible. Figure 6 indicates that heating of PSII core complexes led to changes in the state of the preparations, which led to the destruction of the complex. The first one started at $33.5\text{ }^{\circ}\text{C}$ (Figure 6C), the second at $43.5\text{ }^{\circ}\text{C}$, the third at $56.5\text{ }^{\circ}\text{C}$, and the fourth at $66.7\text{ }^{\circ}\text{C}$ (Figure 6B). To simplify the discussion, we call these transitions A ($43.5\text{ }^{\circ}\text{C}$), B1 ($56.5\text{ }^{\circ}\text{C}$), C ($66.7\text{ }^{\circ}\text{C}$) (the nomenclature accepted in [26] was used), and A2 ($33.5\text{ }^{\circ}\text{C}$) (the nomenclature accepted in [28] was used). A sharp increase in the Q-factor value at $33.5\text{ }^{\circ}\text{C}$ indicated changes in the size or shape of the particles in the suspension. Indeed, using differential scanning calorimetry and gel electrophoresis separation, the dissociation of the proteins of the PSII water-oxidizing complex (without protein denaturation) was previously demonstrated [28]. Transition A observed in Figure 6B may be due to the degradation of the WOC [26,28,97], PSII core monomerization [98], and damage to the PSII acceptor side with denaturation of 43 kDa, 28 kDa, and 22 kDa proteins [27,28]. Moreover, lipid phase transitions may occur at this temperature, but in PSII core complexes, its contribution should be minimal [99]. Practically the full disappearance of transition A in pre-illuminated particles may be due to photodamage to the corresponding components of PSII [100,101]. As has been shown earlier, transition B1 reflects denaturation of the proteins of the PSII core (47 kDa, D1 and D2) [26,28,102]. Surprisingly, the amplitude of transition B1 in pre-illuminated samples was several times higher than in untreated PSII core complexes. Now, it is difficult to explain what this is connected with. It is likely that 10 min of illumination of the samples in an

environment containing oxygen caused significant damage to PSII's components. These damages can lead to a change in the properties of polypeptides due to their oxidation by reactive oxygen species [51,75,103–117], as well as the formation of cross-links between them [118–123]. On the one hand, the damages can reduce the thermal stability of the peptides, and on the other hand, cross-links can increase it. It is likely that during photoinhibition, additional covalent cross-links of the protein complex appear, leading to an increase in thermal stability. As a result of photoinhibition, the process of the destruction of the complex observed in the control samples at 44–52 °C may be shifted to the region of 57–60 °C and occurs in a much narrower temperature range. Note that the properties of untreated and pre-illuminated particles became similar after ≈ 60 °C. It is believed that transition C (identical in both types of preparations) reflects the degradation of minor light-harvesting complexes [20]. However, the PSII core complexes used in the present work did not contain these components. We assumed that transition C may reflect the denaturation of the PsbO protein of the water-oxidizing complex. Phase transitions of PsbO were observed at temperatures from 68 °C to 76 °C, depending on the presence of calcium and manganese ions and the pH of the medium [124].

Our data show that a fixed-length ultrasonic interferometry can be used not only to analyze simple compounds, but also complex ones, such as photosynthetic complexes isolated from the thylakoid membranes of higher plants, as well as the kinetics of heat- and photo-induced processes in them.

5. Conclusions

The present study showed the high sensitivity of the ultrasonic spectrometer we developed. The spectrometer proved itself in studies of both relatively simple compounds (for example, solutions of deuterium oxide and liposomes) and extremely complex multicomponent enzymes (for example, the core complexes of photosystem II of higher plants). Thus, the present study demonstrates that a fixed-length ultrasonic spectrometer can be applied in further investigations to determine the kinetics of heat- and photo-induced damage to biological objects.

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References

1. Losada-Pérez, P.; Jiménez-Monroy, K.; Van Grinsven, B.; Leys, J.; Janssens, S.; Peeters, M.; Glorieux, C.; Thoen, J.; Haenen, K.; De Ceuninck, W. Phase transitions in lipid vesicles detected by a complementary set of methods: Heat-transfer measurements, adiabatic scanning calorimetry, and dissipation-mode quartz crystal microbalance. *Phys. Status Solidi* **2014**, *211*, 1377–1388. [CrossRef]
2. Hazel, J.R.; McKinley, S.J.; Gerrits, M.F. Thermal acclimation of phase behavior in plasma membrane lipids of rainbow trout hepatocytes. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **1998**, *275*, R861–R869. [CrossRef] [PubMed]
3. Hazel, J.R.; Williams, E.E. The role of alterations in membrane lipid composition in enabling physiological adaptation of organisms to their physical environment. *Prog. Lipid Res.* **1990**, *29*, 167–227. [CrossRef]
4. Melchior, D.L.; Steim, J.M. Lipid-Associated Thermal Events in Biomembranes. In *Progress in Surface and Membrane Science*; Cadenhead, D.A., Danielli, J.F., Eds.; Elsevier: Amsterdam, The Netherlands, 1979; Volume 13, pp. 211–296.
5. Sandermann, H. Regulation of membrane enzymes by lipids. *Biochim. Biophys. Acta (BBA)—Rev. Biomembr.* **1978**, *515*, 209–237. [CrossRef]

6. Leonard, J.; Lee, C.; Apperson, A.; Dills, S.; Saier, M. The role of membranes in the transport of small molecules. In *Organization of Prokaryotic Cell Membranes*; CRC Press: Boca Raton, FL, USA, 2018; pp. 1–52.
7. Bondar, A.-N.; Lemieux, M.J. Reactions at Biomembrane Interfaces. *Chem. Rev.* **2019**, *119*, 6162–6183. [[CrossRef](#)]
8. Finkelstein, A.V.; Ptitsyn, O. *Protein Physics: A Course of Lectures*; Elsevier: Amsterdam, The Netherlands, 2016.
9. Mohammadi, P.; Aranko, A.S.; Lemetti, L.; Cenev, Z.; Zhou, Q.; Virtanen, S.; Landowski, C.P.; Penttilä, M.; Fischer, W.J.; Wagermaier, W.; et al. Phase transitions as intermediate steps in the formation of molecularly engineered protein fibers. *Commun. Biol.* **2018**, *1*, 86. [[CrossRef](#)]
10. Shin, Y.; Brangwynne, C.P. Liquid phase condensation in cell physiology and disease. *Science* **2017**, *357*, eaaf4382. [[CrossRef](#)]
11. Jiang, H.; Wang, S.; Huang, Y.; He, X.; Cui, H.; Zhu, X.; Zheng, Y. Phase Transition of Spindle-Associated Protein Regulate Spindle Apparatus Assembly. *Cell* **2015**, *163*, 108–122. [[CrossRef](#)]
12. Zhang, H.; Elbaum-Garfinkle, S.; Langdon, E.M.; Taylor, N.; Occhipinti, P.; Bridges, A.A.; Brangwynne, C.P.; Gladfelter, A.S. RNA Controls PolyQ Protein Phase Transitions. *Mol. Cell* **2015**, *60*, 220–230. [[CrossRef](#)]
13. Fersht, A.R. From the first protein structures to our current knowledge of protein folding: Delights and scepticisms. *Nat. Rev. Mol. Cell Biol.* **2008**, *9*, 650–654. [[CrossRef](#)]
14. Boeynaems, S.; Alberti, S.; Fawzi, N.L.; Mittag, T.; Polymenidou, M.; Rousseau, F.; Schymkowitz, J.; Shorter, J.; Wolozin, B.; Van Den Bosch, L.; et al. Protein Phase Separation: A New Phase in Cell Biology. *Trends Cell Biol.* **2018**, *28*, 420–435. [[CrossRef](#)] [[PubMed](#)]
15. Kharakoz, D.P. On the possible physiological role of the “liquid-solid” phase transition in biological membranes. *Uspekhi Biol. Biol. Khimii* **2001**, *41*, 333–364.
16. Neupane, S.; Cordoyiannis, G.; Renner, F.U.; Losada-Pérez, P. Real-time monitoring of interactions between solid-supported lipid vesicle layers and short-and medium-chain length alcohols: Ethanol and 1-pentanol. *Biomimetics* **2019**, *4*, 8. [[CrossRef](#)] [[PubMed](#)]
17. Yang, S.T.; Kreutzberger, A.J.B.; Lee, J.; Kiessling, V.; Tamm, L.K. The role of cholesterol in membrane fusion. *Chem. Phys. Lipids* **2016**, *199*, 136–143. [[CrossRef](#)] [[PubMed](#)]
18. Subczynski, W.K.; Pasenkiewicz-Gierula, M.; Widomska, J.; Mainali, L.; Raguz, M. High Cholesterol/Low Cholesterol: Effects in Biological Membranes: A Review. *Cell Biochem. Biochem. Biophys.* **2017**, *75*, 369–385. [[CrossRef](#)]
19. Privalov, P.L. Scanning microcalorimeters for studying macromolecules. *Pure Appl. Chem.* **1980**, *52*, 479–497. [[CrossRef](#)]
20. Shi, H.; Xiong, L.; Yang, K.-y.; Tang, C.-q.; Kuang, T.-y.; Zhao, N.-m. Protein secondary structure and conformational changes of photosystem II during heat denaturation studied by Fourier transform-infrared spectroscopy. *J. Mol. Struct.* **1998**, *446*, 137–147. [[CrossRef](#)]
21. Au-Durowoju, I.B.; Au-Bhandal, K.S.; Au-Hu, J.; Au-Carpick, B.; Au-Kirkitaдзе, M. Differential Scanning Calorimetry—A Method for Assessing the Thermal Stability and Conformation of Protein Antigen. *JoVE* **2017**, e55262. [[CrossRef](#)]
22. Privalov, P.L. Stability of proteins small globular proteins. *Adv. Protein Chem.* **1979**, *33*, 167–241.
23. Privalov, P.L.; Gill, S.J. Stability of protein structure and hydrophobic interaction. *Adv. Protein Chem.* **1988**, *39*, 191–234.
24. Donovan, J.W. Scanning calorimetry of complex biological structures. *Trends Biochem. Sci.* **1984**, *9*, 340–344. [[CrossRef](#)]
25. Nolan, W.G.; Hopkins, H.P.; Kalini, S.A.M. Differential scanning calorimetric investigation of pea chloroplast thylakoids and thylakoid fractions. *Arch. Biochem. Biophys.* **1992**, *297*, 19–27. [[CrossRef](#)]
26. Krumova, S.B.; Todinova, S.J.; Dobrikova, A.G.; Taneva, S.G. Differential scanning calorimetry of photosynthetic membranes: Resolving contributions of the major photosynthetic complexes to the sequential thermal transitions. *Trends Photochem. Photobiol.* **2011**, *12*, 37–51.
27. Thompson, L.K.; Sturtevant, J.M.; Brudvig, G.W. Differential scanning calorimetric studies of photosystem II: Evidence for a structural role for cytochrome b559 in the oxygen-evolving complex. *Biochemistry* **1986**, *25*, 6161–6169. [[CrossRef](#)] [[PubMed](#)]
28. Thompson, L.K.; Blaylock, R.; Sturtevant, J.M.; Brudvig, G.W. Molecular basis of the heat denaturation of photosystem II. *Biochemistry* **1989**, *28*, 6686–6695. [[CrossRef](#)] [[PubMed](#)]
29. Blume, A. Biological calorimetry: Membranes. *Thermochim. Acta* **1991**, *193*, 299–347. [[CrossRef](#)]
30. Mineev, K.S. *Development of NMR Spectroscopy Methods and Their Application to Investigate Oligomerization of Membrane Proteins*; Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry: Moscow, Russia, 2019.
31. Krivanek, R.; Jeworrek, C.; Czeslik, C.; Winter, R. Composition Fluctuations in Phospholipid-Sterol Vesicles—A Small-angle Neutron Scattering Study. *Z. Phys. Chem.* **2008**, *222*, 1679–1692. [[CrossRef](#)]
32. Krivanek, R.; Dau, H.; Haumann, M. Enthalpy Changes during Photosynthetic Water Oxidation Tracked by Time-Resolved Calorimetry Using a Photothermal Beam Deflection Technique. *Biophys. J.* **2008**, *94*, 1890–1903. [[CrossRef](#)]
33. Neupane, S.; De Smet, Y.; Renner, F.U.; Losada-Pérez, P. Quartz crystal microbalance with dissipation monitoring: A versatile tool to monitor phase transitions in biomimetic membranes. *Front. Mater.* **2018**, *5*, 46. [[CrossRef](#)]
34. Bryant, C.M.; McClements, D.J. Ultrasonic spectroscopy study of relaxation and scattering in whey protein solutions. *J. Sci. Food Agric.* **1999**, *79*, 1754–1760. [[CrossRef](#)]
35. McClements, D.J. Ultrasonic measurements in particle size analysis. *Encycl. Anal. Chem.* **2000**, 5581–5588.
36. Corredig, M.; Alexander, M.; Dagleish, D.G. The application of ultrasonic spectroscopy to the study of the gelation of milk components. *Food Res. Int.* **2004**, *37*, 557–565. [[CrossRef](#)]
37. Taylor, T.M.; Davidson, P.M.; Bruce, B.D.; Weiss, J. Ultrasonic spectroscopy and differential scanning calorimetry of liposomal-encapsulated nisin. *J. Agric. Food Chem.* **2005**, *53*, 8722–8728. [[CrossRef](#)] [[PubMed](#)]

38. Kertész, I.; Nagy, D.; Baranyai, L.; Pásztor-Huszár, K.; Varsányi, K.; Le Phuong Nguyen, L.; Felföldi, J. Development of a Novel Ultrasonic Spectroscopy Method for Estimation of Viscosity Change during Milk Clotting. *Molecules* **2021**, *26*, 5906. [CrossRef]
39. Sarvazyan, A.P. Ultrasonic velocimetry of biological compounds. *Annu. Rev. Biophys. Biophys. Chem.* **1991**, *20*, 321–342. [CrossRef]
40. Eggers, F.; Funck, T. Ultrasonic measurements with milliliter liquid samples in the 0.5–100 MHz range. *Rev. Sci. Instrum.* **1973**, *44*, 969–977. [CrossRef]
41. Sarvazyan, A.P.; Kharakoz, D.P. Small Volume Differential Interferometer for Velocity and Absorption Measurements of Ultrasound. *Prib. Tekhn. Eksper.* **1981**, 203–206.
42. Kharakoz, D.P. *Investigation of the State and Phase Transitions of Protein Molecules and Lipid Membranes by Measuring the Volume and Compressibility of Solutions*; Institute of Theoretical and Experimental Biophysics of Russian Academy of Sciences: Moscow, Russia, 1995.
43. Blumenfeld, L.A. *Problems of Biological Physics*; Nauka: Moscow, Russia, 1974; p. 335.
44. Astashev, M.; Belosludtsev, K.; Kharakoz, D. Method for digital measurement of phase-frequency characteristics for a fixed-length ultrasonic spectrometer. *Acoust. Phys.* **2014**, *60*, 335–341. [CrossRef]
45. Dubinin, M.V.; Astashev, M.E.; Penkov, N.V.; Gudkov, S.V.; Dyachenko, I.A.; Samartsev, V.N.; Belosludtsev, K.N. Effects of Phospholipase A2 Inhibitors on Bilayer Lipid Membranes. *J. Membr. Biol.* **2016**, *249*, 339–347. [CrossRef]
46. Belosludtsev, K.N.; Belosludtseva, N.V.; Agafonov, A.V.; Astashev, M.E.; Kazakov, A.S.; Saris, N.-E.L.; Mironova, G.D. Ca²⁺-dependent permeabilization of mitochondria and liposomes by palmitic and oleic acids: A comparative study. *Biochim. Biophys. Acta (BBA) Biomembr.* **2014**, *1838*, 2600–2606. [CrossRef]
47. MacDonald, R.C.; MacDonald, R.I.; Menco, B.P.M.; Takeshita, K.; Subbarao, N.K.; Hu, L.-r. Small-volume extrusion apparatus for preparation of large, unilamellar vesicles. *Biochim. Biophys. Acta (BBA) Biomembr.* **1991**, *1061*, 297–303. [CrossRef]
48. Preparing Large, Unilamellar Vesicles by Extrusion (LUVET). Available online: <https://avantilipids.com/tech-support/liposome-preparation/luvet> (accessed on 13 September 2022).
49. Drazenovic, J.; Wang, H.; Roth, K.; Zhang, J.; Ahmed, S.; Chen, Y.; Bothun, G.; Wunder, S.L. Effect of lamellarity and size on calorimetric phase transitions in single component phosphatidylcholine vesicles. *Biochim. Biophys. Acta (BBA) Biomembr.* **2015**, *1848*, 532–543. [CrossRef] [PubMed]
50. van Leeuwen, P.J.; Nieveen, M.C.; van de Meent, E.J.; Dekker, J.P.; van Gorkom, H.J. Rapid and simple isolation of pure photosystem II core and reaction center particles from spinach. *Photosynth. Res.* **1991**, *28*, 149–153. [CrossRef] [PubMed]
51. Khorobrykh, A. Hydrogen Peroxide and Superoxide Anion Radical Photoproduction in PSII Preparations at Various Modifications of the Water-Oxidizing Complex. *Plants* **2019**, *8*, 329. [CrossRef]
52. Ford, R.; Evans, M. Isolation of a photosystem 2 preparation from higher plants with highly enriched oxygen evolution activity. *FEBS Lett.* **1983**, *160*, 159–164. [CrossRef]
53. Lichtenthaler, H.K. Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes. In *Methods in Enzymology*; Elsevier: Amsterdam, The Netherlands, 1987; Volume 148, pp. 350–382.
54. Meister, A.; Kerth, A.; Blume, A. Interaction of sodium dodecyl sulfate with dimyristoyl-sn-glycero-3-phosphocholine monolayers studied by infrared reflection absorption spectroscopy. A new method for the determination of surface partition coefficients. *J. Phys. Chem. B* **2004**, *108*, 8371–8378. [CrossRef]
55. López, O.; Cócera, M.; Pons, R.; Azemar, N.; De la Maza, A. Kinetic studies of liposome solubilization by sodium dodecyl sulfate based on a dynamic light scattering technique. *Langmuir* **1998**, *14*, 4671–4674. [CrossRef]
56. Hildebrand, A.; Beyer, K.; Neubert, R.; Garidel, P.; Blume, A. Temperature dependence of the interaction of cholate and deoxycholate with fluid model membranes and their solubilization into mixed micelles. *Colloids Surf. B Biointerfaces* **2003**, *32*, 335–351. [CrossRef]
57. Juan-Colás, J.; Dresser, L.; Morris, K.; Lagadou, H.; Ward, R.H.; Burns, A.; Tear, S.; Johnson, S.; Leake, M.C.; Quinn, S.D. The Mechanism of Vesicle Solubilization by the Detergent Sodium Dodecyl Sulfate. *Langmuir* **2020**, *36*, 11499–11507. [CrossRef]
58. Deo, N.; Somasundaran, P. Effects of sodium dodecyl sulfate on mixed liposome solubilization. *Langmuir* **2003**, *19*, 7271–7275. [CrossRef]
59. Jafari, M.; Mehrnejad, F.; Rahimi, F.; Asghari, S.M. The molecular basis of the sodium dodecyl sulfate effect on human ubiquitin structure: A molecular dynamics simulation study. *Sci. Rep.* **2018**, *8*, 1–15. [CrossRef] [PubMed]
60. Lillie, M.; Gosline, J. Effects of lipids on elastin's viscoelastic properties. *Biopolym. Orig. Res. Biomol.* **2002**, *64*, 127–138. [CrossRef] [PubMed]
61. Anosov, A.A.; Smirnova, E.Y.; Korepanova, E.A.; Shogenov, I.M. The effects of SDS at subsolubilizing concentrations on the planar lipid bilayer permeability: Two kinds of current fluctuations. *Chem. Phys. Lipids* **2019**, *218*, 10–15. [CrossRef] [PubMed]
62. Inoue, T.; Yanagihara, S.-i.; Misono, Y.; Suzuki, M. Effect of fatty acids on phase behavior of hydrated dipalmitoylphosphatidylcholine bilayer: Saturated versus unsaturated fatty acids. *Chem. Phys. Lipids* **2001**, *109*, 117–133. [CrossRef]
63. Ortiz, A.; Gómez-Fernández, J.C. A differential scanning calorimetry study of the interaction of free fatty acids with phospholipid membranes. *Chem. Phys. Lipids* **1987**, *45*, 75–91. [CrossRef]
64. Eynaudi, A.; Díaz-Castro, F.; Bórquez, J.C.; Bravo-Sagua, R.; Parra, V.; Troncoso, R. Differential effects of Oleic and Palmitic Acids on Lipid Droplet-Mitochondria interaction in the hepatic cell line HepG2. *Front. Nutr.* **2021**, *8*, 775382. [CrossRef]
65. Saitta, F.; Motta, P.; Barbiroli, A.; Signorelli, M.; La Rosa, C.; Janaszewska, A.; Klajnert-Maculewicz, B.; Fessas, D. Influence of Free Fatty Acids on Lipid Membrane–Nisin Interaction. *Langmuir* **2020**, *36*, 13535–13544. [CrossRef]

66. Saitta, F.; Signorelli, M.; Fessas, D. Dissecting the effects of free fatty acids on the thermodynamic stability of complex model membranes mimicking insulin secretory granules. *Colloids Surf. B Biointerfaces* **2019**, *176*, 167–175. [\[CrossRef\]](#)
67. Halstenberg, S.; Heimbürg, T.; Hianik, T.; Kaatz, U.; Krivanek, R. Cholesterol-induced variations in the volume and enthalpy fluctuations of lipid bilayers. *Biophys. J.* **1998**, *75*, 264–271. [\[CrossRef\]](#)
68. Umena, Y.; Kawakami, K.; Shen, J.-R.; Kamiya, N. Crystal structure of oxygen-evolving photosystem II at a resolution of 1.9 Å. *Nature* **2011**, *473*, 55–60. [\[CrossRef\]](#)
69. Loll, B.; Kern, J.; Saenger, W.; Zouni, A.; Biesiadka, J. Lipids in photosystem II: Interactions with protein and cofactors. *Biochim. Biophys. Acta (BBA) Bioenerg.* **2007**, *1767*, 509–519. [\[CrossRef\]](#) [\[PubMed\]](#)
70. Ferreira, K.N.; Iverson, T.M.; Maghlaoui, K.; Barber, J.; Iwata, S. Architecture of the photosynthetic oxygen-evolving center. *Science* **2004**, *303*, 1831–1838. [\[CrossRef\]](#) [\[PubMed\]](#)
71. Zouni, A.; Witt, H.-T.; Kern, J.; Fromme, P.; Krauss, N.; Saenger, W.; Orth, P. Crystal structure of photosystem II from *Synechococcus elongatus* at 3.8 Å resolution. *Nature* **2001**, *409*, 739–743. [\[CrossRef\]](#) [\[PubMed\]](#)
72. Klimov, V.; Shafiev, M.; Allakhverdiev, S. Photoinactivation of the reactivation capacity of photosystem II in pea subchloroplast particles after a complete removal of manganese. *Photosynth. Res.* **1990**, *23*, 59–65. [\[CrossRef\]](#)
73. Jegerschoeld, C.; Virgin, I.; Styring, S. Light-dependent degradation of the D1 protein in photosystem II is accelerated after inhibition of the water splitting reaction. *Biochemistry* **1990**, *29*, 6179–6186. [\[CrossRef\]](#)
74. Telfer, A.; De Las Rivas, J.; Barber, J. β -Carotene within the isolated Photosystem II reaction centre: Photooxidation and irreversible bleaching of this chromophore by oxidised P680. *Biochim. Biophys. Acta (BBA) Bioenerg.* **1991**, *1060*, 106–114. [\[CrossRef\]](#)
75. Yanykin, D.; Khorobrykh, A.; Zastrizhnaya, O.; Klimov, V. Interaction of molecular oxygen with the donor side of photosystem II after destruction of the water-oxidizing complex. *Biochemistry* **2014**, *79*, 205–212. [\[CrossRef\]](#)
76. Theg, S.M.; Filar, L.J.; Dilley, R.A. Photoinactivation of chloroplasts already inhibited on the oxidizing side of Photosystem II. *Biochim. Biophys. Acta (BBA) Bioenerg.* **1986**, *849*, 104–111. [\[CrossRef\]](#)
77. Rao, N.; Mehra, S.; Bridges, J.; Venkatraman, S. Experimental point spread function of FM pulse imaging scheme. *Ultrason. Imaging* **1995**, *17*, 114–141. [\[CrossRef\]](#)
78. Tobocman, W.; Driscoll, D.; Shokrollahi, N.; Izatt, J. Free of speckle ultrasound images of small tissue structures. *Ultrasonics* **2002**, *40*, 983–996. [\[CrossRef\]](#)
79. Amirmazlaghani, M.; Amindavar, H. Wavelet domain Bayesian processor for speckle removal in medical ultrasound images. *IET Image Process.* **2012**, *6*, 580–588. [\[CrossRef\]](#)
80. Gan, T.; Hutchins, D.; Billson, D.; Schindel, D. The use of broadband acoustic transducers and pulse-compression techniques for air-coupled ultrasonic imaging. *Ultrasonics* **2001**, *39*, 181–194. [\[CrossRef\]](#)
81. Iizuka, Y. High signal-to-noise ratio ultrasonic testing system using chirp pulse compression. *Insight* **1998**, *40*, 282–285.
82. Moreau, A.; Lévesque, D.; Lord, M.; Dubois, M.; Monchalain, J.-P.; Padioleau, C.; Bussière, J. On-line measurement of texture, thickness and plastic strain ratio using laser-ultrasound resonance spectroscopy. *Ultrasonics* **2002**, *40*, 1047–1056. [\[CrossRef\]](#)
83. Potter, M.; Dixon, S.; Morrison, J.; Suliamann, A. Development of an advanced multimode automatic ultrasonic texture measurement system for laboratory and production line application. *Ultrasonics* **2006**, *44*, e813–e817. [\[CrossRef\]](#)
84. Leemans, V.; Destain, M.-F. Ultrasonic internal defect detection in cheese. *J. Food Eng.* **2009**, *90*, 333–340. [\[CrossRef\]](#)
85. Hæggström, E.; Luukkala, M. Ultrasound detection and identification of foreign bodies in food products. *Food Control* **2001**, *12*, 37–45. [\[CrossRef\]](#)
86. Létang, C.; Piau, M.; Verdier, C.; Lefebvre, L. Characterization of wheat-flour–water doughs: A new method using ultrasound. *Ultrasonics* **2001**, *39*, 133–141. [\[CrossRef\]](#)
87. Cho, B.K.; Irudayaraj, J. A noncontact ultrasound approach for mechanical property determination of cheeses. *J. Food Sci.* **2003**, *68*, 2243–2247. [\[CrossRef\]](#)
88. Rao, N. Investigation of a pulse compression technique for medical ultrasound: A simulation study. *Med. Biol. Eng. Comput.* **1994**, *32*, 181–188. [\[CrossRef\]](#)
89. Gan, T.; Hutchins, D.; Billson, D. Preliminary studies of a novel air-coupled ultrasonic inspection system for food containers. *J. Food Eng.* **2002**, *53*, 315–323. [\[CrossRef\]](#)
90. Benedito, J.; Carcel, J.; Clemente, G.; Mulet, A. Cheese maturity assessment using ultrasonics. *J. Dairy Sci.* **2000**, *83*, 248–254. [\[CrossRef\]](#)
91. Benedito, J.; Simal, S.; Clemente, G.; Mulet, A. Manchego cheese texture evaluation by ultrasonics and surface probes. *Int. Dairy J.* **2006**, *16*, 431–438. [\[CrossRef\]](#)
92. El Kadi, Y.A.; Moudén, A.; Faiz, B.; Maze, G.; Decultot, D. Ultrasonic monitoring of fish thawing process optimal time of thawing and effect of freezing/thawing. *Acta Sci. Pol. Technol. Aliment.* **2013**, *12*, 273–281.
93. Kargerová, A.; Pekař, M. High-Resolution Ultrasonic Spectroscopy Study of Interactions between Hyaluronan and Cationic Surfactants. *Langmuir* **2014**, *30*, 11866–11872. [\[CrossRef\]](#)
94. Cardona, T.; Sedoud, A.; Cox, N.; Rutherford, A.W. Charge separation in Photosystem II: A comparative and evolutionary overview. *Biochim. Biophys. Acta (BBA) Bioenerg.* **2012**, *1817*, 26–43. [\[CrossRef\]](#)
95. Guskov, A.; Kern, J.; Gabdulkhakov, A.; Broser, M.; Zouni, A.; Saenger, W. Cyanobacterial photosystem II at 2.9-Å resolution and the role of quinones, lipids, channels and chloride. *Nat. Struct. Mol. Biol.* **2009**, *16*, 334–342. [\[CrossRef\]](#)
96. Mizusawa, N.; Wada, H. The role of lipids in photosystem II. *Biochim. Biophys. Acta (BBA) Bioenerg.* **2012**, *1817*, 194–208. [\[CrossRef\]](#)

97. Shutilova, N.; Semenova, G.; Klimov, V.; Shnyrov, V. Temperature-induced functional and structural transformations of the photosystem II oxygen-evolving complex in spinach subchloroplast preparations. *Biochem. Mol. Biol. Int.* **1995**, *35*, 1233–1243.
98. Lípová, L.; Krchňák, P.; Komenda, J.; Ilík, P. Heat-induced disassembly and degradation of chlorophyll-containing protein complexes in vivo. *Biochim. Biophys. Acta (BBA) Bioenerg.* **2010**, *1797*, 63–70. [[CrossRef](#)]
99. Kirchhoff, H.; Borinski, M.; Lenhert, S.; Chi, L.; Büchel, C. Transversal and lateral exciton energy transfer in grana thylakoids of spinach. *Biochemistry* **2004**, *43*, 14508–14516. [[CrossRef](#)] [[PubMed](#)]
100. Vass, I. Molecular mechanisms of photodamage in the Photosystem II complex. *Biochim. Biophys. Acta (BBA) Bioenerg.* **2012**, *1817*, 209–217. [[CrossRef](#)] [[PubMed](#)]
101. Tyystjärvi, E. Photoinhibition of photosystem II. *Int. Rev. Cell Mol. Biol.* **2013**, *300*, 243–303.
102. De Las Rivas, J.; Barber, J. Structure and thermal stability of photosystem II reaction centers studied by infrared spectroscopy. *Biochemistry* **1997**, *36*, 8897–8903. [[CrossRef](#)] [[PubMed](#)]
103. Okamura, M.; Satoh, K.; Isaacson, R.; Feher, G. Evidence of the primary charge separation in the D1/D2 complex of Photosystem II from spinach: EPR of the triplet state. *Prog. Photosynth. Res.* **1987**, *1*, 379–381.
104. Telfer, A.; Barber, J.; Evans, M. Oxidation-reduction potential dependence of reaction centre triplet formation in the isolated D1/D2/cytochrome b-559 photosystem II complex. *FEBS Lett.* **1988**, *232*, 209–213. [[CrossRef](#)]
105. Telfer, A.; Bishop, S.M.; Phillips, D.; Barber, J. Isolated photosynthetic reaction center of photosystem II as a sensitizer for the formation of singlet oxygen. Detection and quantum yield determination using a chemical trapping technique. *J. Biol. Chem.* **1994**, *269*, 13244–13253. [[CrossRef](#)]
106. Ananyev, G.; Klimov, V. Photoproduction bound hydrogen peroxide in subchloroplast preparations of photosystem II. *Dokl AN SSSR* **1988**, *298*, 1007–1011.
107. Ananyev, G.; Wydrzynski, T.; Renger, G.; Klimov, V. Transient peroxide formation by the manganese-containing, redox-active donor side of photosystem II upon inhibition of O₂ evolution with lauroylcholine chloride. *Biochim. Biophys. Acta (BBA) Bioenerg.* **1992**, *1100*, 303–311. [[CrossRef](#)]
108. Ananyev, G.; Renger, G.; Wacker, U.; Klimov, V. The photoproduction of superoxide radicals and the superoxide dismutase activity of Photosystem II. The possible involvement of cytochrome b559. *Photosynth. Res.* **1994**, *41*, 327–338. [[CrossRef](#)]
109. Wydrzynski, T.; Vänngård, T. H₂O₂ formation by photosystem II. *Biochim. Biophys. Acta (BBA) Bioenerg.* **1989**, *973*, 23–28. [[CrossRef](#)]
110. Klimov, V.; Ananyev, G.; Zastryzhnaya, O.; Wydrzynski, T.; Renger, G. Photoproduction of hydrogen peroxide in Photosystem II membrane fragments: A comparison of four signals. *Photosynth. Res.* **1993**, *38*, 409–416. [[CrossRef](#)] [[PubMed](#)]
111. Kruk, J.; Strzałka, K. Dark reoxidation of the plastoquinone-pool is mediated by the low-potential form of cytochrome b-559 in spinach thylakoids. *Photosynth. Res.* **1999**, *62*, 273–279. [[CrossRef](#)]
112. Khorobrykh, S.; Ivanov, B. Oxygen reduction in a plastoquinone pool of isolated pea thylakoids. *Photosynth. Res.* **2002**, *71*, 209–219. [[CrossRef](#)] [[PubMed](#)]
113. Khorobrykh, S.A.; Khorobrykh, A.A.; Yanykin, D.V.; Ivanov, B.N.; Klimov, V.V.; Mano, J.i. Photoproduction of catalase-insensitive peroxides on the donor side of manganese-depleted photosystem II: Evidence with a specific fluorescent probe. *Biochemistry* **2011**, *50*, 10658–10665. [[CrossRef](#)] [[PubMed](#)]
114. Pospíšil, P.; Šnyrychová, I.; Kruk, J.; Strzałka, K.; Nauš, J. Evidence that cytochrome b 559 is involved in superoxide production in photosystem II: Effect of synthetic short-chain plastoquinones in a cytochrome b 559 tobacco mutant. *Biochem. J.* **2006**, *397*, 321–327. [[CrossRef](#)]
115. Pospíšil, P. Production of reactive oxygen species by photosystem II. *Biochim. Biophys. Acta (BBA) Bioenerg.* **2009**, *1787*, 1151–1160. [[CrossRef](#)]
116. Pospíšil, P. Molecular mechanisms of production and scavenging of reactive oxygen species by photosystem II. *Biochim. Biophys. Acta (BBA) Bioenerg.* **2012**, *1817*, 218–231. [[CrossRef](#)]
117. Yanykin, D.; Khorobrykh, A.; Terentyev, V.; Klimov, V. Two pathways of photoproduction of organic hydroperoxides on the donor side of photosystem 2 in subchloroplast membrane fragments. *Photosynth. Res.* **2017**, *133*, 129–138. [[CrossRef](#)]
118. Barbato, R.; Friso, G.; Ponticos, M.; Barber, J. Characterization of the Light-induced Cross-linking of the α -Subunit of Cytochrome b559 and the D1 Protein in Isolated Photosystem II Reaction Centers (*). *J. Biol. Chem.* **1995**, *270*, 24032–24037. [[CrossRef](#)]
119. Henmi, T.; Yamasaki, H.; Sakuma, S.; Tomokawa, Y.; Tamura, N.; Shen, J.-R.; Yamamoto, Y. Dynamic Interaction between the D1 protein, CP43 and OEC33 at the lumenal side of photosystem II in spinach chloroplasts: Evidence from light-induced cross-Linking of the proteins in the donor-side photoinhibition. *Plant Cell Physiol.* **2003**, *44*, 451–456. [[CrossRef](#)] [[PubMed](#)]
120. Ishikawa, Y.; Nakatani, E.; Henmi, T.; Ferjani, A.; Harada, Y.; Tamura, N.; Yamamoto, Y. Turnover of the aggregates and cross-linked products of the D1 protein generated by acceptor-side photoinhibition of photosystem II. *Biochim. Biophys. Acta (BBA) Bioenerg.* **1999**, *1413*, 147–158. [[CrossRef](#)]
121. Salter, A.; De Las Rivas, J.; Barber, J.; Andersson, B. On the molecular mechanisms of light induced D1 protein degradation. *Res. Photosynth.* **1992**, *4*, 395–402.
122. Singh, A.K.; Singhal, G. Formation of cross-linking between photosystem 2 proteins during irradiation of thylakoid membranes at high temperature. *Photosynthetica* **1999**, *36*, 213–223. [[CrossRef](#)]

-
123. Seidler, A. Intermolecular and Intramolecular Interactions of the 33-kDa Protein in Photosystem II. *Eur. J. Biochem.* **1996**, *242*, 485–490. [[CrossRef](#)]
 124. Shutova, T.; Nikitina, J.; Deikus, G.; Andersson, B.; Klimov, V.; Samuelsson, G. Structural dynamics of the manganese-stabilizing protein effect of pH, calcium, and manganese. *Biochemistry* **2005**, *44*, 15182–15192. [[CrossRef](#)]