Long-time oscillations in correlation of lysozyme solutions and the effects of antagonistic salt in external electric field light scattering

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Abstract
Lysozyme is a ubiquitous protein and enzyme that prevents the bacterial infections and maintains the catalytic balance in majority of biological fluids. However, it often causes problems at higher concentrations. In particular, the build up of lysozyme engaged with other protein interactions initiates severe disorders in most mammalian cells, such as the formation of harmful aggregates in the nervous system and the loss of connectivity in rheumatism. To understand such complex behaviors with respective to the catalytic activity of the enzyme, the lysozyme solution and the effect of hydrophobic antagonistic salt (NaBPh4) are explored in-vitro, in their relaxation behaviors. Here, we used, both normal dynamic light scattering and home-built in situ AC external electric field light scattering. As results, the fast and slow-mode (with stretched) relaxations are observed in the conventional dynamic light scattering (without an electric field). The results are particularly noteworthy under a low electric field, with robust long-time oscillations, in the scattered intensity correlation function. In addition, the effective interactions are explored by varying the ionic strength of the antagonistic salt: The oscillations are less pronounced, but still clearly represent the ‘underdamped’ motions. Overall, reductions of the relaxations are shown with the applied electric field, with a maximum relaxation occurring at 1mM. In contrast, monotonic decreases of the relaxation rates are shown above 10 mM. Thus, the lysozyme exhibits a charged carrier, responding to ultimate low-frequency oscillations in the scattered correlations. The fit function of long-time oscillation in correlation is presented by an alternating cosine function with a phase, which is related to its possible dynamic electrophoretic mobility.

1. Introduction

The structure and functions of lysosomes and lysosomal enzyme activities are varied from the primary to oligomeric structures, keeping the ligand-binding proteins [1], and constitutes of connective tissues in majority of mammalian cells [2]. Lysozyme has quite a similar structure with α-lactalbumin [3], abundant in cartilage cells and biological fluids (as tears, sweat and saliva etc). It acts on cataotonic characters in molecular interactions for the adaptive regulations in tissue-specific expressions [1]. As an example, lysosomes are acidic cytoplasmic vacuoles that contain hydrolytic enzymes for sorting hormone clearance and activation, and the extracellular matrix degradation and transmembrane signal transduction. Lysosomal storage diseases (LSDs) are common for the metabolic disorders caused by deficient activity of lysosomal enzyme in the intracellular proteins localized by membrane-bound vesicles, and in particular the reticuloendothelial system [1, 4]. The complexity of LSD is further related to central nervous system (CNS) for accumulation of the neurodegenerative cells, as demyelination and inflammation [5]. There, the enzyme transport vehicles are based on the transferrin receptor, facilitating receptor-mediated transcytosis across the blood-brain barrier (BBB) [6]. Other example is a rare

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tumor of the bone-related one is chordoma, with the vacuolization for large lysosomal mass found in physiological evidences\(^7\).

Another aspect of important function for lysozyme is extracting and isolating plasmid DNA, which can be also harmful in the case of instability for the soluble human lysozyme, forming the amyloid fibrillogenesis \(^8\). Thus, in general, the kinetics and melting transition temperatures of denatured states are directly relevant to amyloid fibers formation. The molecular dynamics simulation \(^9\) are reported for the interactions of lysozyme-SDS complexes, by the hydrophilic and hydrophobic residues.

In addition, various studies on lysozyme activities are reported as follows: Hydrolytic actions of synthetic lysozymes are characterized by the calorimetric assay\(^10\), as well with the thermokinetic Hofmeister effect, in which the anion salt affects with the polarizability of anions to some extents of the low salinity \(^11\). Thermokinetic behaviors are also shown in the solubility and a coexistence curve in the liquid-liquid transitions, across the critical temperature and concentration \(^12\). Temperature-dependent solubility of lysozyme crystals is monotonically increased with the concentration, at high pH values for different NaCl salts \(^13\). Experimental phase diagram for various states of lysozyme in the NaCl salt solution (with morphologies) are shown in the equilibrium, as the crystals, gel, and transient aggregates \(^14\), suggesting that a short-ranged attractive interaction plays a key role in the lysozyme phase behaviors.

For the interactions, concentration dependent diffusion of the lysozyme solution leading to lysozyme crystals is measured by photon correlation spectroscopy, explained by both repulsive and attractive forces, and inter-played by the interaction parameter \(^15\). Several contributions of interactions can be considered, such as via second-virial, the hydrodynamic Oseen, short-, and the long-, and the dipolar terms To simplify those interactions, an effective attractive potential is introduced, and characterized by a Hamaker constant, describing the dispersion force, balanced as the London-van der Waals attraction of two spheres in the molecular scales \(^16\). In practice, interactions of lysozyme can be measured by varying the concentrated electrolyte solutions in DLS, validating the interaction parameter of attractive Hamaker constants \(^17\). However, so far, there is no work of the lysozyme solution, by applying an external electric field, to explore the field-induced microscopic dynamics and effective interactions.

This paper discusses the microscopic relaxation dynamics of lysozyme solutions, by applying alternating low-electric field frequencies. The kinetic behaviors are provided in the \textit{in situ} electric field small angle light scattering. Interestingly, the lysozyme solution has shown ultimately slow relaxations of hydrophobic antagonistic salt (NaBPh4).

2. Experimental details

The preparation of samples and experimental methods are described: We start here with the measurement of normal DLS for a pure lysozyme (concentration of 10 mg/mL) that is performed without an electric-field, followed by the descriptions of \textit{in situ} electric field cell and the vertically aligned (dynamic) light scattering setup. A series of aqueous solutions of antagonistic salt with varying concentration is first prepared. To each of these solutions we added a volume of stock solution of 10 mg/mL lysozyme, such that the final volume corresponds to the desired lysozyme concentration (0.25 mg/mL and 0.5 mg/mL) and the final antagonistic NaBPh4 salts. The Debye screening length of the samples vary from 0.43 nm to 30.4 nm. The scattering wavevector is a fixed at the value of 1.8 \(\mu\text{m}^{-1}\) for varying the electric field frequencies, in the scattered intensity auto-correlation functions.

2.1. Sample preparation

Lysozyme solution is the commercially purchased one from the Sigma-Aldrich (Lot number L3790) \(^18\), as the concentration of 10 mg/mL lysozyme, extracted from chicken egg white (in 25 mM sodium acetate pH 4.5 with 50 percent glycerol), and the molecular weight of single-chain 14.3 kDa, at the optimum pH 9.2. This lysozyme is typically used for the purification of both DNA and protein from bacteria, in the enzymatic lysis of microbial cells \(^19,\ 20\). It also hydrolyzes the beta \textendash\ 1, 4 glycosidic bond between N-acetylglycosamine and N-acetylmuramic acid in the polysaccharide backbone of peptidoglycan present in bacterial cell walls. Lysozyme consists of a single chain polypeptide containing 129 amino acid residues, cross-linked with 4 disulfide bridges \(^21\), shown in figure 1(c). Then the formation of a charge-transfer complex can be occurred by the surface-active agents, such as sodium dodecyl sulfate, sodium dodecanate, and dodecyl alcohol \(^22\). Other compounds of these types also inhibit lysozyme provided that the carbon chain present at least 12 or more carbons in length \(^23\), by N-acetylglycosamine (NAG) and lactone analogs of peptidoglycan \(^24\). NaBPh4 is the antagonistic salt, composed of Na\(^+\) (hydrophilic) and BPh4\(^-\) (hydrophobic) part, called sodium tetraphenylborate (NaBPH4) that is useful to tuning the dynamic mechanical properties, for varying the storage modulus with salt concentration from the molecular structure up to the thermodynamic glass transition \(^25\).
2.2. Normal dynamic light scattering of pure lysozyme solution, much stretched in the slow mode relaxation without an electric field

The relaxations of commercially available lysozyme solution (of a 10 mg/ml) are measured, in the absence of an electric field, under the normal DLS. The concentration is low enough to be an optically transparent solution, and no indication of visible crystals forming, after the measurements. The scattered intensity correlations of the lysozyme solution (of 10 mg/ml) are shown in figure 3; for comparing the 1st day, as a fresh state (up), and the 2nd day, a potential denatured state (down), as a function of the scattering wave vector. The duration time at a single wavevector is 3600 sec, in the consecutive time-series, for the wavevector of 10–30 μm⁻¹. As one can see clearly, the ‘substantially’ stretched slow modes are appeared in the large-time window. The solid red lines are the fits of two mode relaxation for normalized correlation functions in figure 3; one is fast-mode, and the other is slow-mode that stretched. The fitting function for the intensity auto-correlation is used as

$$g_2(t) - 1 = B + A_f \exp(-2\Gamma_f t) + A_s \exp(-2\Gamma_s t),$$  \hspace{1cm} (1)

where \(\Gamma, A, \text{ and } B\) is the relaxation rate (or a damping constant), amplitude, and background, respectively. The origin of the slow component of the intensity correlation functions is most probably slight aggregation of the lysozyme and/or a slight amount contamination of a relatively large sizes (R) from the commercially obtained

![Figure 1. The molecular structures of (a) lysozyme (Reproduced with permission from [26]), and (b) hydrophobic antagonistic salt, sodium tetraphenylborate (NaBPh₄). Here in this work, both the pure lysozyme and the mixtures of antagonistic salt. (c) A long lysozyme provided that the carbon chain present is at least 12 or more carbons in length, inhibited by N-acetylglucosamine (NAG) and lactone analogs of peptidoglycan, purchased from the Sigma-Aldrich Co. This Sodium tetraphenylborate has been obtained by the author(s) from the Wikimedia website [https://en.wikipedia.org/wiki/Sodium_tetraphenylborate], where it is stated to have been released into the public domain. It is included within this article on that basis [27].](image)
lysozyme. Since the scattered intensity from aggregates varies R6, even a small number of aggregates is already visible in dynamic light scattering experiments.

From the fast relaxing mode of the intensity correlation functions without the addition of antagonistic salt, we find a hydrodynamic radius of 6.9 ± 0.3 nm. This relatively large radius as compared to the known hydrodynamic radius of about 2 nm as determined from light scattering from very dilute solutions is attributed to attractive interactions between the proteins, which leads to a decrease of the collective diffusion coefficient. The concentration of lysozyme in the corresponding light scattering experiment is 10 mg/ml, which corresponds to a volume fraction of about 0.1, that is sufficiently large that such attractive forces come into play. Other useful general information of principles and applications of light scattering can be found in refs. [28, 29]. The stretching parameter is found to be far stretched as 0.25 < β < 0.35 (much lower than the stretching of 0.7 < β < 0.8 in normal mode), for extended long time tails in the correlation functions. These slow-time tail relaxations, in the absence of an electric-field, indicate that the slow mode of lysozyme solution alone undergoes non-monotic relaxations.

Diffusion consonants are shown in the inset of figure 3, obtained from the slope of dispersion relation, i.e., $D = \Gamma_0/q^2$, for corresponding to each mode by $D_{\text{fast}} = \Gamma_0/q^2$, and $D_{\text{slow}} = \Gamma_0/q^2$, as well the stretched slow mode is as $D_{\text{stretch}} = D_{\text{slow}}^\gamma$. Similar values of the diffusion constants are found for both samples, as $D_{\text{fast}} = 25.7 - 26.0 \mu \text{m}^2/\text{s}$, $D_{\text{slow}} = 7.8 - 8.2 \mu \text{m}^2/\text{s}$, and $D_{\text{stretch}} = 2.06 - 2.12 \mu \text{m}^2/\text{s}$.

Here, much notable stretched slow mode relaxations are observed ‘substantially’ stable enough in the long-time window, by varying the wavevectors. Such stretched slow modes are presented visible in the scattered field amplitude of the correlation functions, which is resulted in the reduced slow mode diffusion constant, as the lowest one in the inset of figure 3.

### 2.3. In-situ electric cell and vertically aligned small-angle electric-field dynamic light scattering

To apply an electric-field in the light scattering, a homemade optically transparent electrical cell is built for both imaging through a microscope, and the small angle dynamic light scattering [30]. A custom-designed ITO coated float glass (from Przisions Glas und Optik GmbH, CEC500S) is made of dimensions 40 × 70 mm² with a thickness of 0.7 mm. The ITO layer has a high visible light transmission (90 percent) at 633 nm and the coating thickness is 15 nm. Figure 2(a) shows a schematic side view of the in situ electric cell, where the sample is contained in between two ITO coated glass slides. The ITO coatings are electrically connected to a function generator (Avtech model AV-151G-B) via electronic pins (whose dimensions are 50 × 35 mm²). In the experiments presented here, the sinusoidally varying electric potential is applied to the electrodes. The two glass slides are held apart by an insulating rectangular PTFE teflon ring film-sparcer (Armbrrecht and Matthes GmbH, AR5038 and AR5038GP), within which the sample is contained. Finally, teflon tape is used to fixate the glass slides together and to prevent sample evaporation. Typical sample volume is about 300 μl.

The optical fiber is placed on an arm that is connected to the goniometer [28].
Relatively shorter range of the wavevector (1–8 μm⁻¹) is probed in small angle electric-field dynamic light scattering (SAeDLS), as compared to the normal DLS. A special feature of this SAeDLS is the true scattering angle ought to be calibrated from the goniometer angle, by the Snell’s law, for taking care of the refractive index.

Figure 2. Experimental setup of in situ small angle electric-field dynamic light scattering (SAeDLS): (a) The in situ electric cell and the scattering geometry through the cell, where the external electric field is applied to the vertical alignment to distinguish the anisotropic wavevector components, as the perpendicular and parallel. (b) Real view of the SAeDLS, in which the red arrows are the propagating direction of red Laser beam, passing through the in situ electric cell, indicated as horizontal yellow line. (c) The comparison of scattering wavevectors corresponding to scattering angles, between the SAeDLS, and the conventional normal DLS setup. Note that smaller scattering wavevector in SAeDLS is featured with the anisotropic components of the wavevector that are not accessible in the normal DLS setup.
matching condition on the surface of the ITO glass substrates. The difference between two angles is insignificant for the scattering angles lower than 20°, as the corresponding perpendicular component of the wave vector as \( q \sim 3.6 \text{ \( \mu \)m}^{-1} \). Figure 2(c) shows the comparison of scattering wavevectors corresponding to scattering angles, between the SAeDLS, and the conventional DLS setup. \textit{Note that the smaller scattering wavevector in SAeDLS is the range of wavevector that not accessible in the conventional normal DLS setup}, which is also beneficial for probing anisotropic components of the scattered lights, in the micron length of correlations.

3. Results of long-time residual oscillations in the correlation under low external electric field

Here, we have the results of field-induced scattered intensity auto-correlation function, to reveal (i) the microscopic relaxations at different waiting time series that are shown by varying the field frequencies, for the 1st day, and 2nd day lysozyme concentration of 10 mg/ml. (ii) Also the diluted lysozyme solutions are measured by varying the ionic strength of a hydrophobic antagonistic salt (NaBP4), to explore the effective interaction between lysozyme and antagonistic salt, under an \textit{in situ} electric field light scattering.

3.1. Ultimate low frequency oscillations in the slow correlation of pure lysozyme solution

Slow relaxation behaviors of a pure lysozyme (for the concentration 10 mg/ml) are measured, under applying the electric field via the SAeDLS, for the fresh (1st day) and the potential denatured (2nd day) samples. In the previous section, the slow-time oscillations are clearly obtained for a pure lysozyme solution in the normal DLS, in the absence of an electric-field (see figure 3). Such existing slow relaxation behaviors are now further explored by a small angle dynamic light scattering (SAeDLS), under a low AC electric-field. By varying the sinusoidal frequencies, for a given small wavevector of 1.8 \( \mu \text{m}^{-1} \), thermal fluctuation of microscopic dynamics for pure lysozymes are measured.

Strikingly different results of the field-induced dynamics are observed in the background of correlation functions, in figures 4 and 5, for the 1st day, and 2nd day lysozyme solution (of 10 mg/ml), respectively. Much coherent and pronounced long-time oscillations are occurred, as the field-induced responses for a microscopic modulation relaxation. Here the left panels are earlier time-series, and the right one is later time-series, for varying the field frequencies. The correlation functions in these figures have been collected over a time span of 600; s. As can be seen, quite coherent oscillations are observed at long times (in the total time, up to 200 min measurement). As compared to the normal DLS, the scattering wavevector for a SAeDLS is far low as the wavevector of \( q \sim 1.8 \text{ \( \mu \)m}^{-1} \), above the times of 100 ms – 10 sec in the normalized correlation functions. Here, the correlation function of a time range of 0.1 ms – 10 ms is ruled out, due to the fast mode relaxation is the constant (as 1) in normalized correlation function. Thus, the normalized correlation functions are shown, above 100 ms in time, where the slow mode is to be highlighted for field-induced oscillations by varying the field frequency.

These frequency-induced oscillations sustain coherently by the modulation of slow relaxations that are ‘underdamped’. This is then explained by lysosomes as charged carrier in an electric field, such that its local mobility as ‘oscillating’ response to an alternating external electric field, at the low scattering wave-vector. By varying the frequency, long-time oscillations in the correlation maintain for given time measurements, coherently shown as the broad in time. Similar tendency appears for the 2nd day sample, since these field-induced oscillations are consistently occurred in the measurement, under a SAeDLS.

An example of actual fit for the normalized correlation function is shown in figure 6(a), for the whole time range, in which the fast time is shown as constant, with the long-time oscillations that are clearly observed in the background. The sample condition is the lysozyme concentration of 10 mg/ml, applied by a frequency of 1 kHz for 2 V/mm. The relaxation of the pure lysozyme can be distinguished, qualitatively, as corresponding to three regimes diffusive relaxation time: as (i) the fast mode for molecular thermal fluctuations (less than 100 \( \mu s \)), (ii) intermediate relaxation (100 \( \mu s \)–400 ms), and (iii) the slow relaxation time for the aggregation of interacting lysozyme with the solvency (400ms–10 s). In practice, here, the slow-mode is assumed to be cooperatively shown in the ultimately low-frequency oscillations, with visible contributions of the amplitude in scattered correlation functions.

Now one can fit the long-time (or the background) oscillations in the correlations, such that including the expression of a cosine oscillating term, multiplied by a single-stretched exponential function, in the scattered intensity correlation. The actual fit function is then used as,

\[
g_{1}(t) \sim B + A_{f} \exp(-2f_{f}t) + A_{s} \exp(-(\Gamma_{s} t)^{3})) \times \cos(C \sin(\Omega t + \phi)),
\]

where \( C, \Omega \) and \( \phi \) are new fit parameters for the background oscillation amplitude, frequency, and the phase retardation, respectively.
From the normalized correlation function in the scattered intensity, fitting is shown by the red line, as the cosine oscillation term with its own amplitude, $C = 7.93$, and an oscillation frequency $\Omega = 3.2 \times 10^{-4}$, and the phase of $\phi = 4.10$. Note that the oscillation frequency $\Omega$ is not much different with the relaxation time of $\Gamma_{\text{slow}} = 4.7 \times 10^{-4}$, in magnitude. This is then understood that the frequency-modulation are possible to occur as the oscillations that are slightly correlated in the collective relaxation. Apparently, the oscillation amplitude $C$ is better-fitted in the normalized intensity correlation function, while the oscillation frequency $\Omega$ is depicted well in the raw correlation in figure 6(b).

Thus, the lysozyme solution (alone) behaves as the strongly correlated system, here, in the long-time oscillation, by low-frequency modulations. This then suggests that the slow dynamics of lysozyme as charge carriers is realized, under an oscillating external electric field, as an 'underdamped' Brownian motion. Thus, it is a further interest to validate the electrostatic interactions of lysozyme solution, by varying the ionic strengths of

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**Figure 3.** Scattered intensity auto-correlations of a lysozyme solution (of 10 mg/ml), for the 1st day (up), and the 2nd day (down), as a function of the scattering wave vectors, under a conventional ALV normal dynamic light scattering. The duration time of each correlation function, at a given wavevector, is 3600sec. The arrow indicates an increase of wavevector. The dispersion relation of corresponding is shown in the inset, with the diffusion constant of the fast, slow mode, and the stretched slow mode, as the slope of dispersion relation, $D = \Gamma / q^2$. 

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hydrophobic antagonistic salt (NaBPh4), under an external electric field, which is discussed in the following subsection.

3.2. Effective interactions of the mixture of lysozyme and the hydrophobic antagonistic salt

Both stabilization and the interaction range of lysozyme solutions are interested, by varying the ionic strength of a hydrophobic antagonistic salt (NaBPh4). Very diluted lysozyme solutions are used under the in situ SAeDLS. For the low concentration of a lysozyme solution (of 0.25 mg/ml), the solution seems to be stable, relatively at the higher ionic strength of antagonistic salt, above a threshold value (of a 100 mM salt). With an increase of the lysozyme concentration (0.5 mg/ml), the threshold value for stability is decreased to an ionic strength (of a 5 mM salt), which implies that apparently effective interactions are relevant. Figure 7 is the comparison of normalized

![Image](https://via.placeholder.com/150)

Figure 4. Typical example of field-induced oscillations in the scattered intensity auto-correlation function, for a fixed small wavevector of 1–8 μm⁻¹ in different waiting time series; for the 1st day (as a fresh sample) lysozyme concentration of 10 mg/ml, by varying the applied field frequencies, as 1 Hz, 100 Hz, and 1 kHz. Left panels are the earlier time-series of 600 sec duration time, and the right is later time-series for the same duration time. Note that the amplitude of oscillations are strongly correlated (and enhanced) at later time-series. Here, the long-time oscillation frequencies are visibly seen, in the time range of 1–10 sec.
auto-scattered intensity correlation function of a very diluted lysozyme concentration (of 0.25 mg/ml) for different antagonistic salt (NaBPh4) concentrations, applied with few electric-field frequencies, at a fixed low field amplitude (of 2.5 V/mm). The wavevector is $q \sim 1.8 \mu m^{-1}$, measured by an in situ small angle dynamic light scattering (SAeDLS). Although the long-time oscillation amplitudes are a bit reduced, but still remained as background oscillations in the correlations. The red circles, in figures 7 and 8, are such background oscillations in the long-time window, for the various field frequencies.

The comparisons of with-and without-electric field normalized auto-scattered intensity correlation function are shown for two diluted lysozyme concentration of 0.25 mg/ml and 0.50 mg/ml; with few low antagonistic salt (NaBPh4) concentrations, applied to an electric-field frequency of 100 Hz and a fixed field low field amplitude (of 2.5 V/mm). The scattering wave vector is of $q \sim 1.8 \mu m^{-1}$, in the in situ small angle dynamic light scattering (SAeDLS). Here, in the presence of an electric field (see the upper panel), the relaxations are shifted to slower, as compared to the absence of a field (lower panel). Rather, well-behaved correlation functions are observed, with still sustained noisy longer-time correlations, when the low electric field is applied.
Also the comparison of normalized auto-scattered intensity correlation functions for two diluted lysozyme concentration of \(0.25 \text{ mg/ml}\) and \(0.50 \text{ mg/ml}\) are shown in figure 8, for three low ionic strengths (of \(0.1 \text{ mM}, 1 \text{ mM},\ and \ 5 \text{ mM}\)) of antagonistic salt. The blue horizontal arrow indicates the signal-to-noise ratio of 0.5 in the correlation function, in which faster relaxation becomes to slower, in an increase of the ionic strength. Such slow mode relaxations are still visible in longer-time correlation, with the ‘reduced’ oscillation amplitude that is slightly decreased with an increase of the ionic strength of a hydrophobic salt.

The summary of the correlation functions, with- and without the field, are provided in figure 9, upper and lower panel, respectively. Notably slower relaxations are obtained in the presence of an electric-field (in the upper of figure 10), as compared to the absence. Although relaxation times can be distinguished by the three regimes, as the fast time (less than 100 ms), intermediate (100 ms-5 sec), and the slow time (5–100 sec), for the simplicity, two dynamical modes are fitted to extract relaxation rates, focused with the slow-mode stretched. The results of relaxation rates are then shown in figure 10, for the diluted lysozyme in an effect of hydrophobic
antagonistic salt (NaBPh4). Ultimately slow oscillations in the background correlation are being dealt separately, in later subsection.

As the results on relaxation rates for the mixture of two diluted lysozyme concentrations, characteristically different features are shown in the relaxations, as a function of the ionic strength of hydrophobic antagonistic salt (NaBPh4): Apparent reduction of the relaxations are shown with the applied electric field (figure 10).

Interestingly, for both samples, a maximum relaxation occurs at 1mM, on the contrary, monotonic decreases of the relaxation rates are shown, above 10 mM, for both with- and without-electric field, in the fast and slow modes (see the thick vertical arrow bars in figure 10). Also lower relaxation rates (see the pink line) are obtained at higher concentration of lysozyme. This is then agreed with results of other system for biological glycolipids (gangliosides), as a dilute solution of ionic micells at a low ionic strength, below 5 mM, while above 10 mM to higher, the diffusion becomes coincide with the predictions [31]. It then mimics a charged colloidal behavior,

**Figure 7.** The comparison of normalized auto-intensity correlation function of a very diluted lysozyme concentration (of 0.25 mg/ml) for different antagonistic salt NaBPh4 concentrations. Here, the electric field amplitude is 2.5 V/mm, with few field-frequencies, at a fixed scattering wavevector of $q \sim 1.8 \mu m^{-1}$), measured by the in situ small angle dynamic light scattering (SAeDLS). The blue horizontal arrow indicates the signal-to-noise ratio of 0.5 in the correlation function. Longer-time correlations are less pronounced as compared to the pure lysozyme, but still present, as highlighted in the red circles.
such that the microscopic dynamics is effectively affected by the concentration of particles for varying the ionic strength.

3.3. Possible explanation of the long-time background oscillations, a field-induced dynamic electrophoretic mobility

More details of long-time slow modulations of electric field-induced oscillations are presented, in figure 11, for the very diluted lysozyme in the mixtures of various ionic strength of antagonistic salt (NaBPh4). Here, the electric field amplitude is 2.5 V/mm, with few field-frequencies, at a fixed scattering wavevector of $q \sim 1.8 \mu m^{-1}$), measured by the in situ small angle dynamic light scattering (SAzDLS). As an increase of lysozyme concentration (on the right panel), slower relaxations are shown, as compared to the lower concentration (on the left). The blue horizontal arrow and the red circles correspond to the same description in figure 7.

*Figure 8.* The comparison of normalized auto-intensity correlation function of two diluted lysozyme concentration of 0.25 mg/ml and 0.50 mg/ml, for 3 low ionic strengths (of 0.1 mM, 1mM, and 5 mM) antagonistic salt (NaBPh4). Here, the electric field amplitude is 2.5 V/mm, with few field-frequencies, at a fixed scattering wavevector of $q \sim 1.8 \mu m^{-1}$), measured by the in situ small angle dynamic light scattering (SAzDLS). As an increase of lysozyme concentration (on the right panel), slower relaxations are shown, as compared to the lower concentration (on the left). The blue horizontal arrow and the red circles correspond to the same description in figure 7.
behavior, in the presence of electric-field, ultimately as the low-frequency response range, below 1–0.01 Hz, in the correlation.

The above can be partly explained by the followings: In a static electric field, double layers of the lysozyme-salt aggregates will screen the electric field over a distance of the order a Debye length, similar to the electrode polarization. To meet the criterion for net electric field in the bulk (of a solution) as zero, the double layers act as charge-stabilized, and the resultant charges within the double layers ought to compensate in the externally applied field. Therefore, due to the finite diffusivity of the salt ions, the double layer of particles could be also ‘finite’, when an oscillatory external field is applied.

Such interplay of the electrostatic interaction energy exists in the solution of intermicellar in an aqueous SDS, demonstrated by DLS and explained by DLVO theory for a repulsive interaction [31, 32]. Therefore, in this work, the long-time background oscillations can be the consequence of a delicate balance between the effective interactions of lysozyme solutions, affected by the hydrophobic antagonist salt, under low-frequency modulations in external electric field. This is then understood by a field-induced dynamic electrophoretic mobility existing in the lysozyme-antagonistic salt aggregate. Furthermore, dynamic electrophoretic mobility can be pronounced, in the case of hydrodynamic slipping via the surface potential in an oscillating electric field [33]. Such dynamic slip is then shown for larger electrostatic Debye screening lengths, varied from 4.3 nm, 9.61 nm, and 30.4 nm, respectively, for 5mM, 1mM, and 0.1 mM NaBPh4, compared to the hydrodynamic radius of lysozyme solution (1.86–2.28 nm) [34]. Therefore, apparently, in the presence of an external AC field, ultimate low frequency-response are observed as the coherent background oscillations for well-behaved ‘underdamped’ motions of the system.

Figure 9. The comparison of with- and without-electric field for the normalized auto-intensity correlation function of two diluted lysozyme concentration of 0.25 mg/ml and 0.50 mg/ml, with three low ionic strengths of antagonist salt (NaBPh4), applied with an electric-field frequency of 100 Hz and a fixed field amplitude (of 2.5 V/mm), and a scattering wavevector of $q \sim 1.8 \mu m^{-1}$, measured by the in situ small angle dynamic light scattering (SAeDLS). The relaxations are shown as shifted to slower in the presence of an electric field (see the upper panel), as compared to the absence of a field (lower panel). Rather, well-behaved correlation functions are observed when the low electric field is applied, with a broader dispersion in relaxations, including the long-time oscillations (whose details are seen in figure 11).
4. Conclusion

We have shown here the microscopic relaxation dynamics of lysozyme solutions (of 10 mg/ml), and the effects of antagonistic salt (NaBPh4), for both without- and with- the low external electric field. Under an alternating low-electric field frequencies, as compared to the normal dynamic light scattering for the pure lysozyme (without an electric-field), striking difference of relaxation behaviors are found as the long-time oscillations in the background of scattered intensity auto-correlation functions. These field-included oscillations can be fitted...
by a cosine function that is related to slow-mode relaxations, for their own oscillating frequency and the phase lags. Visibly pronounced amplitudes of oscillations are shown for longer time-series, which indicates that ultimately low-frequency oscillations are ‘unavoidable’ due to the field-response of an intrinsic nature of lysozyme enzyme activity as the catalytic behaviors, in the low external AC electric field.

The effective interactions of diluted lysozyme solutions are revealed further by varying the ionic strengths of a (hydrophobic) antagonistic salt, under the in situ external electric-field light scattering. The observed non-monotonic relaxation behaviors support the interplay of resulting effective attractive interactions of lysozyme-antagonistic salt mixture, as the short-attraction and a cut-off long-ranged electrostatic repulsive interaction. Also the van der Waals interaction may be relevant, which is often occurred in the cell surfaces and cell-substrate via the surface interactions [35]. As a follow up interest, the specific role of hydrophobic interactions of the lysozyme solution can be explored by the mixture a lysozyme with proteins or the DNA-virus (fd) suspension, by varying the concentration of lysozyme. Finally, we hope that the results are useful for establishing the nature of ultimately slow-dynamics for the lysozyme solution, responded to low external field-frequencies. This then can be used not only to monitor in real-time, but also to build more systematic probes to explore long-time residual oscillations of enzymatic activity in the application to other biological relevance.

Data availability statement

All data that support the findings of this study are included within the article (and any supplementary files).

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