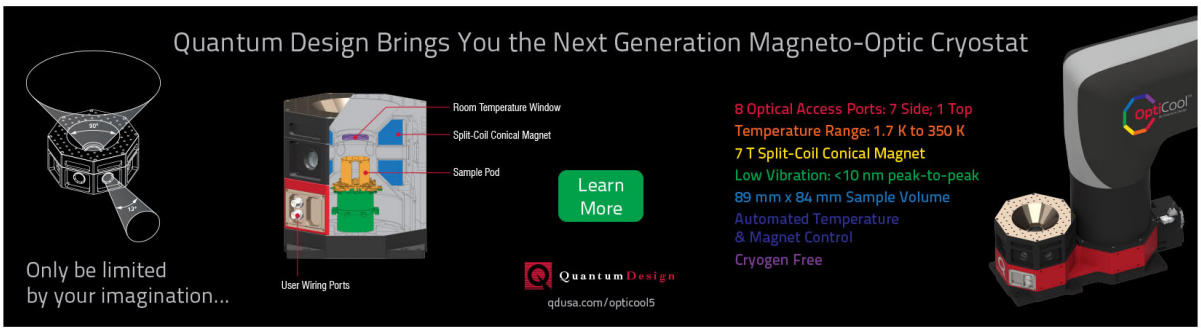
# Pyroelectricity in globular protein lysozyme films

A. Stapleton, M. R. Noor, E. U. Haq, C. Silien, T. Soulimane, and S. A. M. Tofail

Citation: Journal of Applied Physics **123**, 124701 (2018); doi: 10.1063/1.5014029
View online: https://doi.org/10.1063/1.5014029
View Table of Contents: http://aip.scitation.org/toc/jap/123/12
Published by the American Institute of Physics





# Pyroelectricity in globular protein lysozyme films

A. Stapleton,[1,2] M. R. Noor,[2,3] E. U. Haq,[1,2] C. Silien,[1,2] T. Soulimane,[2,3] and S. A. M. Tofail[1,2]
[1]*Department of Physics, University of Limerick, Limerick V94 T9PX, Ireland*
[2]*Bernal Institute, University of Limerick, Limerick V94 T9PX, Ireland*
[3]*Department of Chemical Sciences, University of Limerick, Limerick V94 T9PX, Ireland*

(Received 14 November 2017; accepted 15 March 2018; published online 29 March 2018)

Pyroelectricity is the ability of certain non-centrosymmetric materials to generate an electric charge in response to a change in temperature and finds use in a range of applications from burglar alarms to thermal imaging. Some biological materials also exhibit pyroelectricity but the examples of the effect are limited to fibrous proteins, polypeptides, and tissues and organs of animals and plants. Here, we report pyroelectricity in polycrystalline aggregate films of lysozyme, a globular protein. *Published by AIP Publishing.* https://doi.org/10.1063/1.5014029

## INTRODUCTION

Pyroelectricity is the ability of certain non-centrosymmetric materials to generate a current when they experience a change in temperature over time; the pyroelectric current is proportional to the rate of heating or cooling. All pyroelectric materials also demonstrate piezoelectricity, the ability to generate a charge around their surface when subjected to stress (the direct effect) or conversely, to develop a mechanical strain in response to an electric field (the converse effect).

Both pyroelectricity and piezoelectricity are inherently dependent on crystallographic principles. Piezoelectricity requires that the material will have a non-centrosymmetric crystal structure so there is a net polarization when the material's dimension changes due to a mechanical deformation. Pyroelectric materials have further restrictions, requiring that the material's unit cell has a dipole moment and spontaneous polarization along a unique polar axis.[1] Twenty of the 32 crystal point groups are piezoelectric. Of the 20 piezoelectric point groups, ten are polar and demonstrate pyroelectricity.

While pyroelectricity is common in many solid-state materials, examples of pyroelectricity in biological materials are relatively less studied. Since Lang[2] reported the observation of pyroelectricity in bones and tendons in 1966, a handful of studies have reported pyroelectricity in plant leaves,[3] the thorax of live insects,[4] amino acids,[5] as well as poled[6] and un-poled hydroxyapatite.[7] The pyroelectric coefficient reported for $\gamma$-glycine ($13 \times 10^{-6}$ C m$^{-2}$K$^{-1}$)[5] is more than three times higher than that of tourmaline, illustrating that strong pyroelectricity is present even in the smallest biological building blocks. Until now, however, pyroelectricity in protein crystals has not been reported. Here, we show experimental evidence of the pyroelectric effect in crystalline aggregate films of lysozyme.

Lysozyme is a globular protein found in secretions such as saliva and tears; it is also found abundantly in hen-egg whites. Its function is to protect against infection by breaking down bacteria cell walls. While the dielectric properties of many solid-state pyroelectrics are well known, relatively few studies have investigated the dielectric properties of lysozyme. The dielectric constant of lysozyme ranges between 2 and 80, depending on whether it is in its powder or reconstituted state.[8] An intermediate value of 30 for the dielectric constant is probably the most appropriate,[9] comparable to the dielectric constant of lithium niobate (28.7).[10] Lysozyme can be crystallized in various forms: tetragonal, monoclinic, orthorhombic, hexagonal, and triclinic. Monoclinic crystals of lysozyme belong to crystal point group 2, which is a polar symmetry group.[11] Recently, we have shown experimental evidence of the converse piezoelectric effect in monoclinic crystals of lysozyme using piezoresponse force microscopy.[12] We have also shown that crystalline aggregate films of both monoclinic and tetragonal lysozyme exhibit the conventional direct piezoelectric effect.[13] Based on its polar crystal structure and observations of its piezoelectric behaviour, we hypothesize that monoclinic crystals of lysozyme should demonstrate the pyroelectric effect. Here, we provide the experimental evidence behind such hypothesis.

## EXPERIMENTAL

### Preparation of lysozyme films

Protein crystals are soft and brittle[14] owing to their high water content. Additionally, protein crystals must be kept hydrated in order to maintain their structure. Therefore, handling and electroding protein crystals are extremely challenging. As described earlier (see Ref. 13), we have overcome this challenge by preparing lysozyme as a film on a conductive substrate. Briefly, monoclinic aggregate films of lysozyme were prepared by reconstituting 50 mg of lysozyme powder in 1 ml of sodium acetate (50 mM, pH 4.6). This protein solution was then mixed with 4% (w/v) sodium nitrate in a 1:1 ratio. To prevent the films from cracking during drying, 1 $\mu$l of glycerol (diluted to 50% in ultrapure water) was added to the protein solution. Typically, 100 $\mu$l of the final protein solution was dropcast on the electrode. The protein drop was left to dry overnight in a temperature-controlled room (20 °C), during which the crystallization occurred.

### Electroding aggregate films of lysozyme

In this study, we used two types of electrodes: (i) indium-tin oxide (ITO) coated glass slides and (ii) inter-digitated

electrodes (IDEs). In the first case, the lysozyme solution was dropcast directly on to a piece of ITO-coated glass. Once dry, we placed a second piece of ITO-coated glass on top of the film, which acted as the second electrode. When using the ITO-electrodes, the electroded area was equivalent to the area of the dropcast film (approximately 113 $\mu m^{-1}$). However, as only those lysozyme crystals with their polar axis perpendicular to the electrodes contribute to the pyroelectric effect, the effective electrode area may only be a fraction of this.

In the second case, silver IDE's were screen-printed using a commercial screen-printer (Fino Essemtec, Switzerland). Silver paint (9912-K, ESL, Europe) was pushed through a mesh screen (MCI Precision Screens Ltd., UK) on to an alumina substrate to create the desired IDE features. The screen-printed IDE has twenty fingers of width 0.2 mm and with an inter-digit spacing of 0.2 mm. The IDE's were fired in a furnace at 150 °C for 10 min and then at 850 °C for 30 min. The thickness of the IDEs post firing was 20 $\mu$m. When using the IDE-electrodes, the electrode area is equivalent to the sum of the areas of the lysozyme film between adjacent IDE fingers. After drying, typically the lysozyme films covered 70% of the IDE, thus, the electrode area was approximately 15.9 $\mu m^2$. However, as with the ITO-electrode arrangement, we emphasize that only crystals of lysozyme whose b-axis is perpendicular to the IDE fingers, contribute to the pyroelectric current. Electrical wires were soldered to the IDE bond pads. Then, the electrical wires were connected to the electrometer with crocodile clips. Protein films were created on the IDEs by drop-casting the protein solution directly on to the IDE and drying overnight, as described above.

### Byer-Roundy method of pyroelectric measurement

We used a Byer-Roundy Method to investigate the pyroelectric effect in monoclinic aggregate films of lysozyme. The method is based on the principle that the pyroelectric current is proportional to the rate of change of temperature.[15] A schematic of the pyroelectric measurement system is depicted in Fig. 1.

A Peltier heating stage (Linkam Scientific Instruments Ltd., UK) was used as the heating/cooling source. A Peltier controller (Linkam T95) controlled the set-point temperature and the rate of heating/cooling. The system has a temperature range of −20 °C to 120 °C. The rate of heating/cooling can be varied between 0.1 °C $min^{-1}$ and 20 °C $min^{-1}$. The temperature controller uses an in-built thermocouple to control the temperature of the Peltier stage. To ensure uniform heating and reduce the contribution of tertiary pyroelectricity, a second external thermocouple placed directly on the sample surface recorded accurate measurements of the sample temperature throughout the measurement. The temperature range was selected so that it was well below the temperature at which lysozyme denatures (approximately 79 °C at pH 4.6).[16,17] An electrometer (Keithley 6514) measured the current through the sample. A custom LabVIEW program operated the Peltier controller, thermocouple, and electrometer remotely and facilitated data logging. Measurements were recorded at a rate of one data point per second.

The pyroelectric measurement system was verified with positive and negative controls. The positive control, a commercial sample of poled Polyvinylidene difluoride (PVDF) (Piezotech SAS, France), was electroded by placing it between two pieces of ITO-coated glass. It was heated from 20 °C to 40 °C at a rate of 2 °C $min^{-1}$ and then it was cooled from 40 °C to 20 °C at the same rate. The poled PVDF sample generated a negative pyroelectric current (approximately minus 20 pA) during heating and a positive pyroelectric current during cooling. The pyroelectric coefficient for this sample of poled PVDF was calculated to be approximately 28 $\mu C\,m^{-2}K^{-1}$. This compares well with the literature values of the pyroelectric coefficient of PVDF ($p = 30\,\mu C\,m^{-2}K^{-1}$) poled under similar conditions.[18] The negative control, a piece of plain glass placed between two pieces of ITO-coated glass, did not show any reversal of current polarity when subjected to similar heating and cooling temperature ramps.

## RESULTS AND DISCUSSIONS

### Pyroelectric measurements of lysozyme using ITO-coated electrodes

In this study, we investigated the pyroelectric effect in un-poled films of lysozyme. Although poling may increase the pyroelectric effect in these films, currently there is no known method of poling proteins. The first measurements of monoclinic aggregate films of lysozyme were performed on films sandwiched between ITO-coated glass. The temperature cycle comprised of three stages. During the first stage, the film of monoclinic lysozyme was cooled from 18.1 °C to 5.1 °C. The rate of cooling was set at 0.5 °C $min^{-1}$. As non-uniform heating can adversely affect the measurements, a second external thermocouple directly reads the temperature of the sample throughout the measurement. The actual rate of cooling (measured via the external thermocouple) was determined from the slope of the negative line in Fig. 2(a) to be 0.496 °C $min^{-1}$. The cooling ramp was linear ($R^2$ value of 0.9998). The second stage of the heat cycle kept the temperature fixed at 5.1 °C for 25 min. The purpose of the "hold"

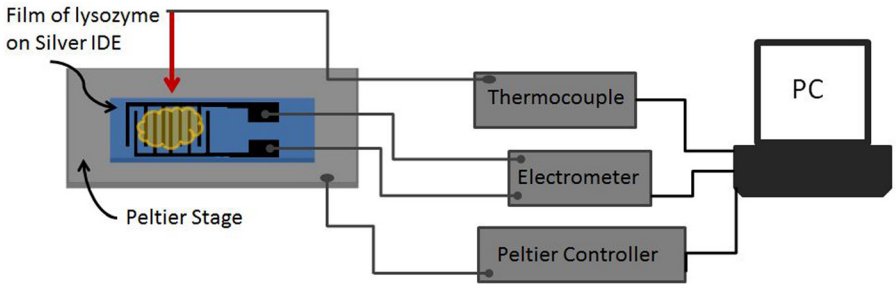



FIG. 1. Schematic of the pyroelectric measurement system. The pyroelectric current through a crystalline aggregate film of monoclinic lysozyme is stimulated by a Peltier heating stage and measured with an electrometer using inter-digitated electrodes.

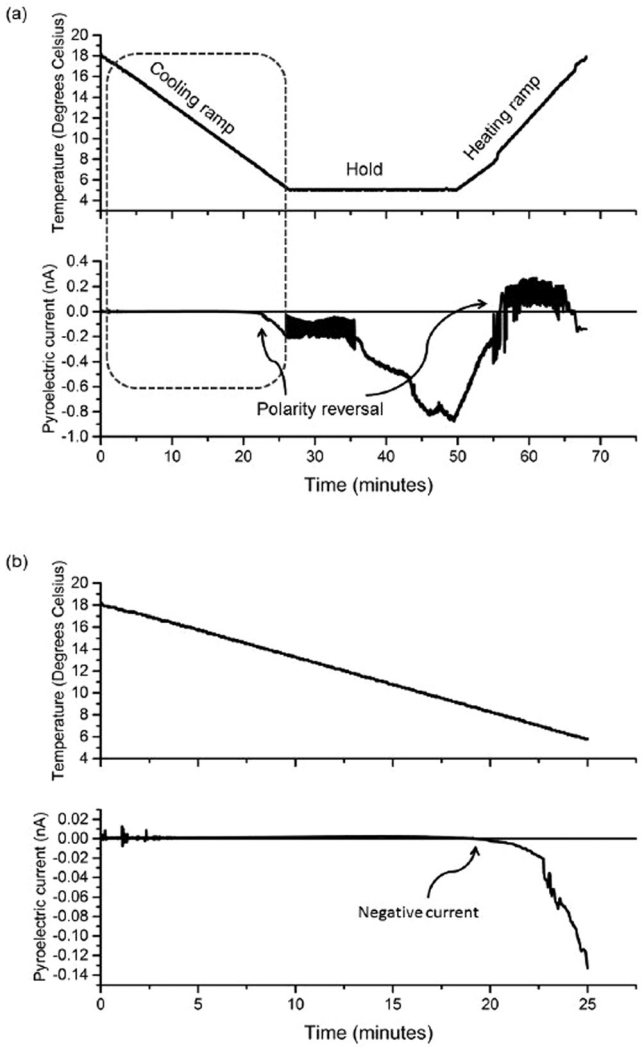



FIG. 2. Pyroelectric effect in a film of monoclinic lysozyme sandwiched between two pieces of ITO-coated glass. (a) The current generated switches polarity becoming negative during the cooling cycle and positive during the heating cycle. (b) Temperature and current generated during the interval indicated by the dashed box in (a) - the current becomes negative at approximately 9 °C.

interval was to allow the sample time to stabilize at the minimum temperature value before initiating the heating ramp. During the third stage, the film was heated from 5.1 °C to 17.9 °C. The rate of heating was set at 0.5 °C min$^{-1}$. The actual rate achieved was slightly higher (0.74 °C min$^{-1}$) because the sample returned to ambient temperature at a rate that the Peltier stage could not fully compensate for. The heating ramp was not completely linear with a few small discontinuities where the Peltier tried to readjust the temperature. Overall, the linearity of the heating cycle is good with an $R^2$ value of 0.9967.

The current through the sample at each stage of the temperature cycle is shown in Fig. 2(a). For clarity, the section of the plot indicated by the dashed box in Fig. 2(a) is rescaled and replotted in Fig. 2(b).

For the first 3.5 min of the measurement, the current through the sample fluctuates from positive to negative. After this, as the temperature decreases, the current is approximately constant; during the time interval between 3.5 min and 19.0 min, the average current is 1.65 pA. At a temperature of 8.6 °C, the current switches polarity and becomes negative. It remains negative for the rest of the cooling ramp.

During the hold interval, the current stays negative and fluctuates greatly, reaching a maximum value of 870 pA. By definition, the current during this hold interval is not pyroelectric in nature as the temperature remains fixed throughout. When the heating ramp is initiated, the current decreases rapidly towards zero and changes to a positive current at approximately 9 °C. The current remains positive until the temperature exceeds approximately 15 °C where it becomes negative again. Although the signal is noisy, the current measured through the monoclinic aggregate film of lysozyme in the temperature interval 5 °C to 9 °C is negative during cooling (approximately 135 nA) and positive during heating (approximately 130 nA). This is indicative of the pyroelectric effect. The fluctuations in current outside of this temperature range are not consistent with pyroelectricity and may be the result of parasitic thermally stimulated currents (TSCs).

The choice of electrode arrangement used in this part of the study may not be most appropriate. We recall that the pyroelectric current develops parallel to the polar axis of the crystal. For monoclinic crystals in point group 2, the polar axis is parallel to the diad axis,[11] i.e., the *b*-axis of the crystal. The monoclinic lysozyme crystals within the film are randomly orientated, with a preference to have their long-axis (polar axis) in the plane of the film. Only crystals within the film, whose polar axis is perpendicular to the electrodes, would have contributed significantly to the measured current. An electrode arrangement that maximizes contact with correctly oriented crystals is preferable. Two possible options are: (i) using a probe station as the electrical contact points or (ii) using an IDE to maximize contact with correctly oriented crystals.

## Pyroelectric measurements of lysozyme using IDE electrodes

In the next part of the study, we carried out pyroelectric measurements on monoclinic aggregate films of lysozyme prepared on IDEs. As the b-axis of the majority of lysozyme crystals lies within the plane film, by using the IDEs, the area of the film with correctly oriented crystals of lysozyme in contact with the electrodes is increased. Since the pyroelectric current is proportional to the area of the sample electroded, it too should be increased.

The film of monoclinic lysozyme was first cooled from 23.9 °C to 8.6 °C. The rate of cooling was set to 5 °C min$^{-1}$. The actual cooling rate as measured by the external thermocouple was 4.17 °C min$^{-1}$. Then, the film was heated at the same rate (actual heating rate was 5.11 °C min$^{-1}$) from 8.6 °C to the maximum temperature of 35.5 °C. A second modification was made for this part of the study; the number of heat cycles was increased to five. By subjecting the sample to several heat cycles, trapped charges that contribute to TSCs are released so that subsequent cycles are less effected.

Figure 3 shows the temperature cycles applied to monoclinic films of lysozyme and the current generated using the

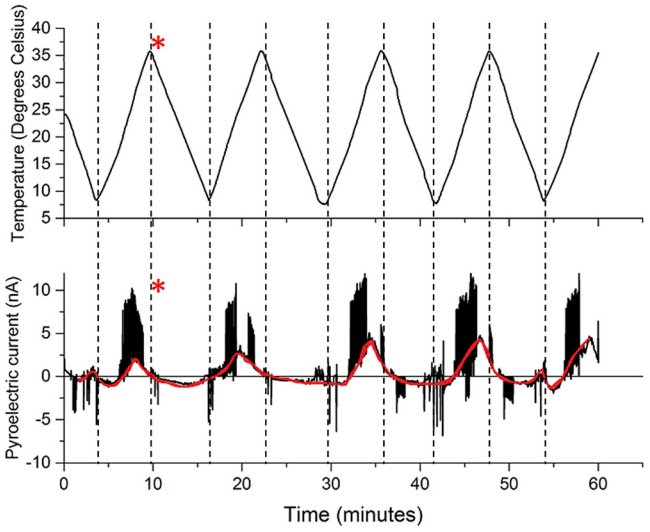



FIG. 3. Pyroelectric effect in a film of monoclinic lysozyme dropcast on an IDE measured over several temperature cycles. The polarity of the pyroelectric current is switched when the heat cycle changes from heating to cooling. The red annotated curve highlights the shape of the current signal.

IDEs. Although the signal contains noise, there is a clear change in polarity of the current in each measurement cycle when the temperature ramp changes from heating to cooling and *vice versa*. The red annotated line in Fig. 3 highlights this trend.

If we examine the current during the heat cycle in more detail, we see that just before the first full cooling cycle (indicated by a red asterisk) is initiated, the current is positive. When the cooling ramp begins, the current immediately becomes negative. It stays negative throughout the cooling cycle. The current only changes polarity to positive when the heating ramp changes from cooling to heating. The current reaches its maximum positive value (taken from the red curve) at 25 °C. As the temperature continues to increase beyond this temperature, the current decreases towards zero, but remains positive throughout the heating cycle.

Throughout the five temperature cycles, the monoclinic film of lysozyme responds in a similar manner to the cycle just described. Apart from the first temperature cycle, the current switches polarity immediately each time the cooling cycle is initiated. In contrast, when the heating cycle is initiated, there is a slight delay before the current switches polarity in all but one of the temperature cycles. Despite this slight lag, the fact that the current switches polarity when the temperature ramp changes from heating to cooling is a hallmark of pyroelectricity.

The current signal is clearly affected by noise; the noise is systematic, occurring most strongly in the same region of each temperature cycle (i.e., during the heating ramp between approximately 19 °C and 30 °C). There is also noise in the current signal during the cooling ramp but to a lesser extent. Because TSCs are irreversible, we believe that the noise is not the result of thermally stimulated currents. The persistence of the noise artefacts over several temperature cycles indicates that they are inherent to the system and could be removed by filtering; we show the unfiltered data here for completeness.

It is also worth noting that the pyroelectric current does not stay at a constant value during either the heating or cooling ramps. Rather, the pyroelectric current appears to experience a relaxation after reaching its maximum value. While the exact mechanisms contributing to this relaxation are unknown at this point, we propose that the presence of glycerol in the lysozyme film may be a contributing factor.

Furthermore, we note that because the lysozyme film is unclamped in the IDE arrangement, it is free to expand and contract during the temperature cycles. Thermal expansion within the film causes a strain to develop that induces a piezoelectric response. This phenomenon is known as secondary pyroelectricity and is considered a true pyroelectric effect. Parasitic effects of tertiary pyroelectricity (false pyroelectricity) were minimized by keeping the temperature ramps linear.

The pyroelectric coefficient $p$ is given by $p = \frac{I}{A\left(\frac{dT}{dt}\right)}$, where $I$ is the pyroelectric current, $A$ is the electrode area, and $\frac{dT}{dt}$ is the rate of change of temperature with respect to time. Given the large uncertainty in calculating precisely the number of crystals within the film correctly oriented with respect to the IDEs, rather than quantifying the pyroelectric coefficient of crystals of lysozyme, we instead quantify the pyroelectric coefficient for the film as a whole. The effective electrode area was $15.9\,\mu\text{m}^{-2}$ and the average $\frac{dT}{dt}$ (during heating and cooling) was $0.0773\,\text{K s}^{-1}$. The pyroelectric current was estimated as the sum of the average current during each heating and cooling cycle (1.8 nA). Using these values, the pyroelectric coefficient of the lysozyme film is $1441 \mp 536\,\mu\text{C m}^{-2}\,\text{K}^{-1}$. The magnitude of the pyroelectric is worth noting, being two orders of magnitude higher than that reported for $\gamma$-glycine. It is of the same order of magnitude as the pyroelectric coefficient of $Pb(ZR_{0.2}Ti_{0.8})O_3$ grown on $SrTiO_3$ substrates ($1900\,\mu\text{C m}^{-1}\,\text{K}^{-1}$).[19] The error is also significant, reflecting the large uncertainty in quantifying the pyroelectric current and effective electrode area.

Further studies (e.g., employing the Chynoweth method[20]) may help to minimize the contribution of noise and thermal relaxation in the pyroelectric signal. Thermal relaxations may be eliminated as the time required for the film to thermally relax may not be met during rapid heating and cooling cycles achieved by a pulse laser. However, quantifying the pyroelectric effect may be difficult because the heat capacity of the lysozyme film and emissivity of the electrodes must be known to determine the change in temperature.[15]

## CONCLUSIONS

Here, we have shown that pyroelectric measurements can be conducted on crystalline aggregate films of monoclinic lysozyme using the Byer-Roundy method. Monoclinic crystals of lysozyme were electroded for such measurements by growing a crystalline aggregate film on either ITO-coated glass or inter-digitated electrodes. In both cases, a pyroelectric current is generated which switches polarity when the temperature ramp changes from heating to cooling or *vice versa*. Crystalline aggregate films of lysozyme prepared on IDEs show a stronger pyroelectric response than those

prepared on ITO-coated glass, perhaps because the effective electrode area of the former is greater. Thermal relaxations during both cooling and heating ramps were observed and call for further studies to uncover their origin. Monoclinic lysozyme crystals are described by polar point group symmetry and thus, these observations conform to the classical description of pyroelectricity.

## ACKNOWLEDGMENTS

Funding from the Irish Research Council EMBARK Postgraduate Scholarship (RS/2012/337) to A.S. is acknowledged.

[1]S. B. Lang, *Modern Bioelectricity* (Marcel Dekker, Inc., 1988).
[2]S. B. Lang, Nature **212**, 704 (1966).
[3]S. B. Lang and H. Athenstaedt, Ferroelectrics **17**, 511 (1977).
[4]H. Athenstaedt and H. Claussen, Biophys. J. **35**, 365 (1981).
[5]V. Lemanov, Ferroelectrics **238**, 211 (2000).
[6]S. Tofail, C. Baldisserri, D. Haverty, J. McMonagle, and J. Erhart, J. Appl. Phys. **106**, 106104 (2009).
[7]S. Lang, S. Tofail, A. Gandhi, M. Gregor, C. Wolf-Brandstetter, J. Kost, S. Bauer, and M. Krause, Appl. Phys. Lett. **98**, 123703 (2011).
[8]S. C. Harvey and P. Hoekstra, J. Phys. Chem. **76**, 2987 (1972).
[9]P. E. Smith, R. M. Brunne, A. E. Mark, and W. F. Van Gunsteren, J. Phys. Chem. **97**, 2009 (1993).
[10]C. R. Bowen, J. Taylor, E. LeBoulbar, D. Zabek, A. Chauhan, and R. Vaish, Energy Environ. Sci. **7**, 3836 (2014).
[11]J. F. Nye, *Physical Properties of Crystals: Their Representation by Tensors and Matrices* (Oxford University Press, 1985).
[12]A. Stapleton, M. R. Noor, T. Soulimane, and S. A. Tofail, *Electrically Active Materials for Medical Devices* (World Scientific, 2016), p. 237.
[13]A. Stapleton, M. Noor, J. Sweeney, V. Casey, A. Kholkin, C. Silien, A. Gandhi, T. Soulimane, and S. Tofail, Appl. Phys. Lett. **111**, 142902 (2017).
[14]S. Tait, E. T. White, and J. D. Litster, Part. Part. Syst. Charact. **25**, 266 (2008).
[15]R. Whatmore, *Characterisation of Ferroelectric Bulk Materials and Thin Films* (Springer, 2014), p. 65.
[16]Y. Zhang and P. S. Cremer, Proc. Natl. Acad. Sci. U.S.A. **106**, 15249 (2009).
[17]A. Cao, G. Wang, Y. Tang, and L. Lai, Biochem. Biophys. Res. Commun. **291**, 795 (2002).
[18]C. Birlikseven, E. Altintaş, and H. Z. Durusoy, J. Mater. Sci.: Mater. Electron. **12**, 601 (2001).
[19]M. Botea, A. Iuga, and L. Pintilie, Appl. Phys. Lett. **103**, 232902 (2013).
[20]A. Chynoweth, J. Appl. Phys. **27**, 78 (1956).