



Full length article

Compression of dry lysozyme targets: The target preparation pressure as a new parameter in protein thin film production by pulsed laser deposition



Catalin Constantinescu^{a,b,c}, Andreea Matei^{a,c}, Marshall Tabetah^d, Maria Dinescu^c, Leonid V. Zhigilei^d, Jørgen Schou^{a,*}

^a DTU Fotonik, Risø Campus, DK-4000 Roskilde, Denmark

^b Université de Limoges, CNRS/IRCEM UMR 7315, F-87068 Limoges, France

^c INFLPR - National Institute for Laser, Plasma and Radiation Physics, RO-077125 Magurele, Bucharest, Romania

^d Department of Materials Science and Engineering, University of Virginia, Charlottesville, VA 22904-4745, USA

ARTICLE INFO

Keywords:

Thin films

PLD

Organics

MAPLE

Lysozyme

ABSTRACT

Film growth of the well-known protein, chicken lysozyme, produced by the dry technique, pulsed laser deposition (PLD), from a compressed powder target has been investigated as a function of the target preparation pressure. PLD is a versatile technique for fabricating high quality films of inorganic materials, but the laser beam will typically produce fragments of molecules in the target and subsequently in the deposited films. We demonstrate that the pressure applied to compact the target prior to the laser irradiation is an important parameter that determines the deposition rate as well as the extent of fragmentation of the deposited molecules. The deposition process was carried out in vacuum using dry targets prepared with compaction pressure in the range 10–160 bar. The residual water in pockets of the lysozyme molecules drives fragments or intact lysozyme out of the target. At the intermediate fluence of 2 J/cm^2 , the deposition rate of the material (fragments or intact molecules) rises from 3 to 9 ng/cm^2 per shot as the compaction pressure increases from 10 to 160 bar. However, the number of intact molecules falls down by almost two orders of magnitude in the same pressure range. This is explained by a stronger cohesion of the target material prepared at higher compression pressure, such that more energy and thus a higher temperature are required for the onset of material ejection. At the highest compression pressure, it means that no intact molecules survive the ejection. The results indicate that there is a pressure range where both a reasonable deposition rate and a considerable fraction of intact molecules in the films can be achieved. These experimental observations are consistent with the results of coarse-grained molecular dynamics simulations, where the fraction of intact lysozyme molecules is observed to vanish as the maximum temperature in the irradiated target increases.

1. Introduction

The deposition of organic materials by laser-assisted methods has become increasingly important for fabrication of thin films of polymers and biomaterials [1–7]. Direct laser-based deposition methods, such as pulsed laser deposition (PLD), have the advantage of not involving any solvent-solute or solute-substrate interactions, which play a significant role in other important deposition methods such as Langmuir-Blodgett and Langmuir-Schaefer deposition techniques [8,9]. On the other hand, the laser-beam interaction with organic molecules can destroy the molecules in the target and produce films with a large fraction of fragments rather than intact molecules [1,5,10–13]. Any application of PLD of organic films requires knowledge of the number of intact

molecules in the films. If this number is sufficiently high one may expect exploitation of active units of organic-inorganic interfaces as constituents of hybrid devices and for the development of heterostructures with unprecedented electric, magnetic and optical properties [14–16]. As discussed below, the fragmentation rate of the molecules in the film strongly depends on the initial target powder compression pressure.

The common laser-assisted technique for depositing organic materials as thin films in vacuum is matrix-assisted pulsed laser evaporation (MAPLE), in which the film material is dissolved in a volatile, frozen matrix [1,17]. The matrix is supposed to absorb laser light and to protect the film molecules from decomposition during laser irradiation. Once the laser beam strikes the target, the matrix material at the point

* Corresponding author.

E-mail address: josc@fotonik.dtu.dk (J. Schou).

<https://doi.org/10.1016/j.apsusc.2019.03.089>

Received 12 October 2018; Received in revised form 5 March 2019; Accepted 11 March 2019

Available online 12 March 2019

0169-4332/ © 2019 Published by Elsevier B.V.

of the laser beam impact is vaporized in an explosive manner and pumped away, while the less volatile film material is collected on a suitable substrate. The conditions for the matrix-free deposition are rarely completely fulfilled, but the method works, and a large variety of films of organic molecules have been produced [17], e.g. ultrastable polymer glasses which could not be produced by other methods [18]. A special advantage of the MAPLE technique is that only a small amount of the film material in the target is consumed by the deposition process, typically 0.5–2% of the frozen target [17,19], which enables precise control over the average thickness of the deposited films.

The main drawback of the MAPLE technique is that even though the substrate is kept in a dry environment, it is far from being a UHV-technique for deposition. During the laser irradiation of the volatile frozen matrix, the pressure increases usually more than two orders of magnitude. The vaporization of a water ice matrix or organic volatile matrices, such as chloroform or toluene, is certainly not compatible with UHV. Any accurate design and fabrication of organic/inorganic heterostructure systems require matrix-free targets or at least a strongly reduced evaporation from a matrix, which can be difficult to achieve [20]. Another drawback of the MAPLE technique is that large chunks of the matrix with embedded film molecules can be transferred to the substrate, resulting in non-uniform coverage featuring complex surface structures resembling deflated balloons and collapsed pipes [21,22].

In the present work, we deposited films by PLD, i.e. irradiating a target of pressed chicken egg white lysozyme powder in a holder without the addition of any matrix. The deposition was performed directly in vacuum with ns-pulses at 355 nm, in a manner similar to earlier work with PLD on lysozyme [5–7,23,24]. Lysozyme is useful as a model protein for deposition in vacuum, since its properties and structure are well-known [5], it is not too heavy (14,307 amu), and the bioactivity can be tested before and after the deposition. Lysozyme is a bactericidal protein [25], but, on the other hand, it also forms harmful layers on artificial surfaces in the human body after denaturation [26].

The ejection mechanism of lysozyme molecules or fragments from a pressed, dry lysozyme target during laser irradiation was investigated by coarse-grained molecular dynamics (MD) simulations for a lysozyme-water system [5]. It was found that ablation from a pure lysozyme system was possible, but that no intact lysozyme molecules were ejected by such a PLD process. The fragmentation processes that drive the ablation simultaneously split all lysozyme molecules into fragments. Surprisingly, however, the MD simulations predict that a minor fraction of water embedded in the lysozyme molecules is capable of driving the ablation process in which intact lysozyme molecules can reach the substrate. In fact, a narrow window of fluences that can lead to the transfer of intact lysozyme molecules to a substrate appears at a water concentration as low as 5 wt%, and this window expands with increasing concentration of water. The conclusion from this study is that “pulsed laser deposition” of intact lysozyme molecules is actually a MAPLE-like process, with residual water from internal pockets serving the role of a matrix, rather than a strict PLD-process [5]. The process is actually referred to as “inverse MAPLE” in Ref. [5], since the ratio of the deposited material to the “matrix” in the target can be as high as 10:1 instead of the typical 1:100 in the conventional MAPLE. In order to avoid confusion with the conventional MAPLE, however, in this paper we will refer to the film deposition through the direct irradiation of a “dry” lysozyme target as “PLD.”

Most of our earlier results for lysozyme deposition have been obtained for the same PLD geometry as described below and with a laser fluence in the range of a few J/cm² at a wavelength of 355 nm [5–7,24]. The surface morphology was studied as well, and it turned out that the surface of the films was covered with micrometer-size grains for PLD performed at a fluence of 2 J/cm², although the dependence on the target compression pressure was not investigated in these studies [6,7,24].

Since it turned out that the results for the deposition and the

decomposition rate strongly depend on the initial target powder compression pressure, we performed an additional quantitative investigation of the effect of the pressure, reported in the present paper. The fraction of intact molecules in films was measured as it is an important quantity that defines the functional behavior of the film. To our knowledge this is the first study focused on the effect of the target preparation pressure in PLD of organic materials.

2. Experimental section

The experimental setup consists of a Nd:YAG laser beam directed at an angle of 45° with respect to normal onto a target located in a vacuum chamber with a base pressure of 5×10^{-5} mbar. The laser operates at 355 nm with a pulse duration of about 6 ns and a fluence of ~ 2 J/cm². The laser beam was focused on the target on an area of 0.017 cm², and a repetition rate of 2 Hz was used. The wavelength of the laser is well above the absorption threshold of the lysozyme molecules in an aqueous solution, i.e. 310 nm, but, nevertheless, pressed dry lysozyme does absorb laser light well at this wavelength [25].

The substrate was positioned at a distance of 60 mm and was either the silver electrode of a quartz crystal microbalance (QCM) or a Si <100> substrate of an area of 7×7 mm². The quartz crystal electrode has a circular 6-mm diameter sensor area, and the accuracy of the deposition measurements is $\sim 1.29 \times 10^{16}$ amu/cm² [27]. Typically, a deposition measurement with the quartz crystal microbalance was carried out for 400–600 shots. As described below, the target was a freshly pressed chicken egg white lysozyme from Sigma Aldrich with molecular weight of 14,307 Da (amu). The target cup had an internal diameter of 17.3 mm and a depth of 7.3 mm. The laser beam was rastered across the target powder to minimize the drilling of holes, and the target was rotated in all the experiments.

The target was prepared by putting fresh lysozyme powder into the target holder, until it was completely filled up (see Fig. 1). The pressing cap on the target holder was then pressed with the screw vice until the desired load was reached. If the powder volume was reduced, additional powder was filled into the holder and the same pressure applied again. The pressure was monitored with a calibrated load sensor FUTEK, model LLB400, and the density ρ was found to increase linearly with pressure p :

$$\rho \text{ (g/cm}^3\text{)} = 0.59 + 1.64 \times 10^{-3} p \text{ (bar)}.$$

After the filling to the desired pressure was completed, the target holder was inserted in the vacuum chamber. The value of 0.59 g/cm³ corresponds to lysozyme powder without any external (compression) pressure.

During the initial experiments, the target surface was kept in a vertical position similar to standard PLD experiments. However, such a target inclination had the unexpected effect that large mm-size chunks fell out of the target when the compression pressure was below 20 bar. Thus, the heating and the subsequent evaporation pressure induced by the laser heating were sufficient to drive loosely bound chunks out of the target. The results presented here are all from target holders oriented with an inclination of 45° to the vertical plane.

The quantitative MALDI (Matrix Assisted Laser Desorption Ionization) analysis was performed on a commercial MALDI TOF-MS system (Bruker Reflex IV MALDI-TOF) equipped with a nitrogen laser ($\lambda = 337$ nm, 3 ns pulse length). The sample preparation was performed according to the procedure described in Ref. [6]: a matrix solution was prepared by mixing 70 mg sinapinic acid with 2 ml acetonitrile and 1 ml water with 0.1% trifluoroacetic acid (TFA). A drop of 0.5 μ l solution was poured onto the lysozyme film, then the sample was transferred onto a MALDI plate and left to dry in vacuum prior to the analysis. Each MALDI spectrum was averaged over 2000 laser shots. The spectra were recorded with the linear TOF in positive ion mode.

The quantitative analysis was carried out in exactly the same way for all values of the compression pressure. The background was

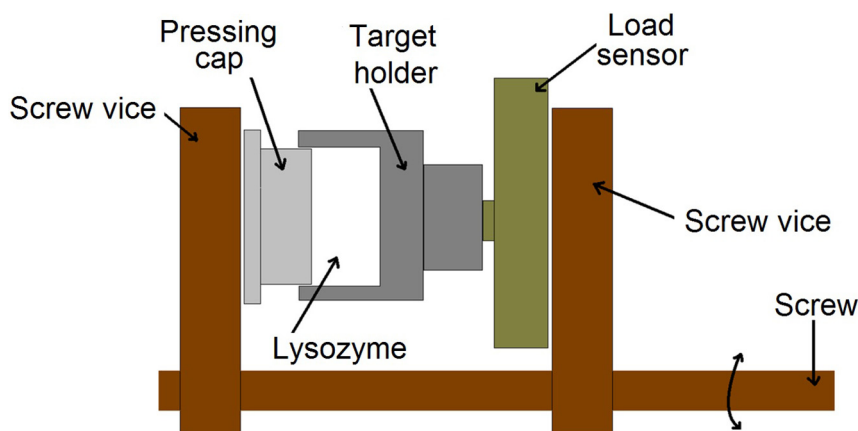


Fig. 1. Schematic of a compression device for target and holder.

subtracted, the same beam setting and number of pulses from the nitrogen laser for the MALDI system were used. Except for the doubly charged ions, there was no structure in the MALDI spectra [5,6], which is in complete agreement with the inverse-MAPLE mechanism proposed in Ref. 5. All MALDI samples were prepared with the same matrix and the same thickness, and the only parameter that was varied, was the preparation pressure of the lysozyme powder of the PLD target.

3. Experimental results

The decrease of the resonance frequency of the QCM during film deposition as a function of the number of pulses is shown in Fig. 2. Each curve has been produced with a fresh load of lysozyme in the target holder. One notes that the slope for all curves decreases with an increasing number of pulses, as also reported in Ref. [7], which reflects the modification of the lysozyme surface during the first 100 pulses. All frequency curves reported in previous articles by the present authors have been recorded for a pressure of 62.5 bar corresponding to a deposition of 6.6 ng/cm² of fragments or intact lysozyme molecules [5–7,24].

The deposition rate as a function of preparation pressure in the range from 8.2 bar up to 132 bar is shown in Fig. 3. Essentially, the deposition rate is increasing monotonically from 2.9 ng/cm² at the

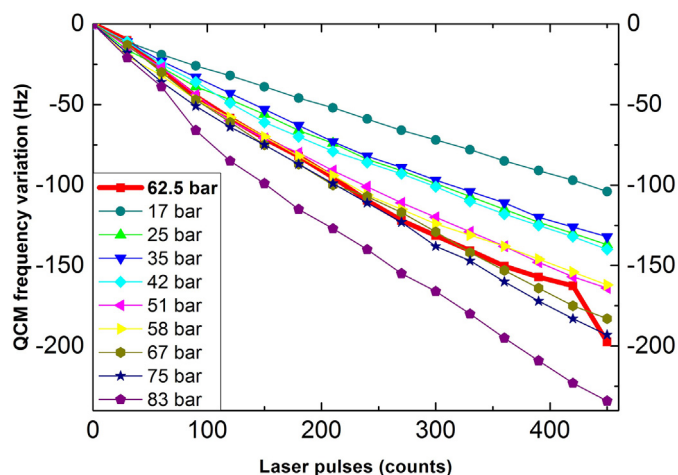


Fig. 2. The decrease in the frequency of the quartz crystal monitor (QCM) as a function of the number of laser pulses, shown for lysozyme targets prepared with different values the compression pressure. The decrease of the frequency corresponds to an increase of the thickness of the lysozyme film (see text). The targets used in earlier publications [5–7,24] were prepared with a pressure of 62.5 bar.

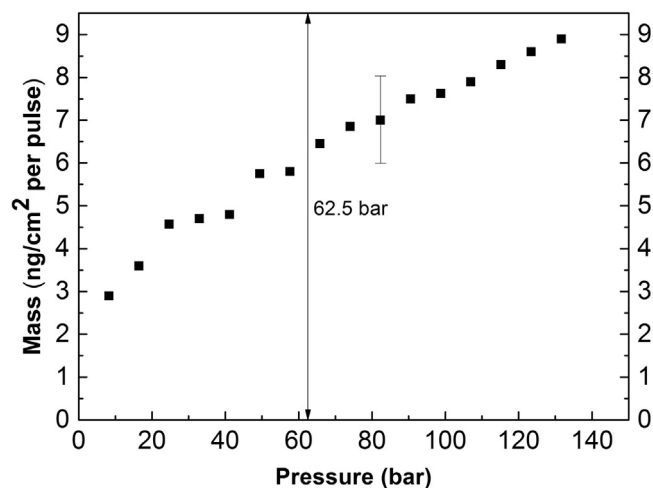


Fig. 3. The lysozyme deposition rate per pulse as a function of compression pressure used in the target preparation. The deposited material can contain both intact lysozyme molecules and molecular fragments. The vertical line marks the pressure value of 62.5 bar used in previous studies reported in Refs. [5–7, 24]. The error bar indicates a representative value of the scattering of the data [5].

lowest pressure up to 8.9 ng/cm² at the highest pressure. The target was mounted with an inclination of 45°, such that the lysozyme did not fall out during the laser irradiation. At the lowest pressure, however, a substantial part (up to a one-half) of the ablated lysozyme was ejected in chunks which could be collected on a horizontally placed plate below the target.

One important point of consideration is the “survival rate” of the lysozyme molecules at high compression pressure. The MALDI peak of lysozyme molecules with the mass centered at (14,307 + 1) Da/charge corresponding to a protonated lysozyme molecule is depicted as a function of the preparation pressure in Fig. 4. Obviously, there is no significant fraction of intact lysozyme molecules at a pressure much above 85 bar. This is demonstrated as well with Fig. 5, in which the area below the peak is plotted for the same values of the preparation pressure as in Fig. 4. At the highest pressure, there are practically no intact molecules transferred to the substrate. The resolution of the measurements (full width at half maximum) is 160 amu, and no features in the spectrum are observed except for a small, unidentified shoulder at 14470 amu. Below the mass range of the main peak at around 14,308 amu shown in Fig. 4, there is a featureless spectrum of broken lysozyme molecules except for the small peak of doubly charged lysozyme molecules mentioned earlier.

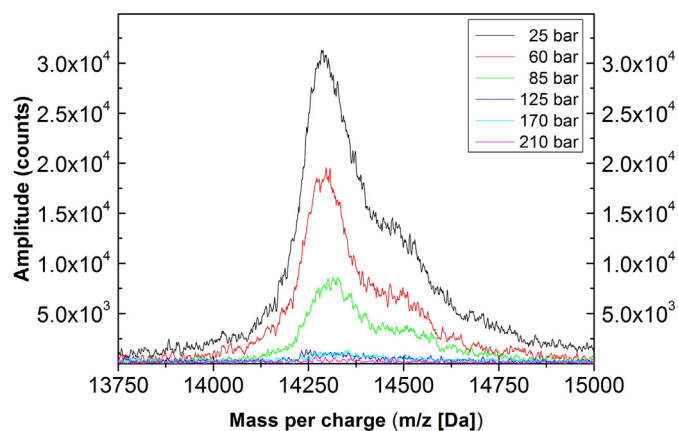


Fig. 4. MALDI signal in the vicinity of the molecular weight of an intact lysozyme molecule, 14,307 Da, measured for films produced from targets prepared with different compression pressure.

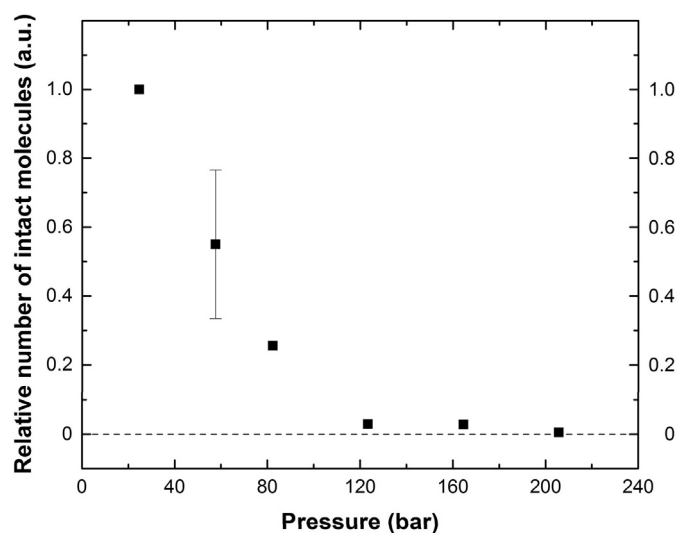


Fig. 5. Integrated signal of intact lysozyme molecules evaluated for the molecular mass interval shown in Fig. 4. The values are normalized by the number of intact molecules obtained for the lowest compression pressure. The error bar indicates a representative value of the scattering of the data [5].

4. Discussion

The influence of the target preparation pressure on the PLD process has only been discussed few times. The compression pressure of targets for PLD is occasionally mentioned, in particular for comparisons between MAPLE and PLD for organic materials [3,4]. In one single case, Smausz et al. explored the influence of the target preparation pressure on the surface roughness parameter in films of bioceramic materials from human teeth [28]. They found that the roughness increased up to a factor of 1.5 when the pressure was increased from 1.5 kbar to 4.5 kbar. This pressure range is substantially higher than the one used in the present study, and we have merely investigated the surface morphology for our standard pressure at 62.5 bar [7].

An important point for the discussion is the direct influence of the pressure on the material properties of lysozyme powder. The density of our target is 0.73 g/cm^3 at 82.3 bar, compared with 1.237 g/cm^3 of lysozyme crystals [29]. The fractional volume change reported for lysozyme crystals is 0.037 for a compression of 1.01 kbar, and any intramolecular structural changes between a pressure of 1 bar and 1000 bar are insignificant [29]. For our targets, the fractional volume reduction is 0.171 for a compression from 82 bar to 165 bar. Our targets

are thus quite porous, and the compression in the present experiment is not expected to induce any molecular fragmentation or conformational changes of the individual molecules. Rather, the compression is likely to reduce/reshape the air and water pockets and increase the internal cohesion in the target.

The ablation under the experimental conditions used in this work has clearly *photothermal* rather than photomechanical nature. This was demonstrated by irradiating a dry lysozyme layer of thickness $100 \mu\text{m}$ between two quartz windows with a low pulse energy. The lysozyme becomes immediately brown and discolored. At the same time, this experiment also provides us with a penetration depth for the laser light of the order of $30 \mu\text{m}$. The relatively small penetration depth indicates that the laser light is efficiently absorbed in the lysozyme target. Since lysozyme in an aqueous solution does not absorb light at 355 nm [30], the absorption is assisted by defects of microcrystals in the powder and subsequently by fragments of the lysozyme.

The simulations reported in Ref. [5] demonstrate that the ejection of intact lysozyme molecules is only possible when the main driving force responsible for the material ejection is the vaporization of water pockets and rapid expansion of water vapor rather than release of the volatile products of thermal decomposition of lysozyme molecules. Indeed, while the release of volatile fragments can lead to the ablation of dry lysozyme target, the simulations predict that no intact lysozyme molecules can survive the ejection process in this case due to the high temperature and extensive pyrolytic decomposition of the molecules. The onset of the explosive vaporization of water, on the other hand, is initiated at lower temperatures, when the molecular decomposition can still be limited or even completely avoided. To illustrate this computational prediction, the maximum values of temperature reached in the top 30-nm-thick surface region of an irradiated lysozyme target containing 10 wt% of residual water are plotted as a function of laser fluence in Fig. 6. It has been established for the model system used in the simulations [5] that the time required for thermally-activated scission of 5% of the chemical bonds in a pure lysozyme material drops from $\sim 14 \text{ ns}$ at a temperature of 2900 K down to $\sim 400 \text{ ps}$ at a temperature of 3700 K . The presence of water, however, enables ablation under conditions where the maximum temperature is still not high enough for thermal decomposition of all lysozyme molecules ejected in the ablation process. As a result, there exists a window of fluence (marked by gray background color in Fig. 6) that can yield the ejection and

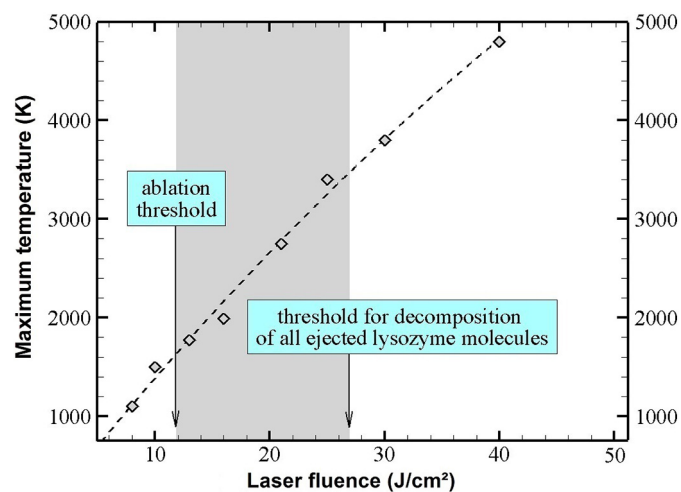


Fig. 6. The maximum values of temperature reached in the top 30-nm-thick surface region of an irradiated lysozyme target containing 10 wt% of residual water as a function of laser fluence predicted in coarse-grained MD simulations [5]. A range of fluences between the thresholds for ablation and decomposition of all lysozyme molecules is highlighted by gray background color. The irradiation at fluences within this range can produce the ejection and deposition of intact molecules.

deposition of intact lysozyme molecules.

Note that direct quantitative matching of threshold fluences required for the ablation of thin lysozyme films considered in the simulations and the bulk targets used in experiments is not possible. Even though the experimental values of heat capacities of water and lysozyme are reproduced by the coarse-grained MD model, and the experimental value of the optical penetration depth is used in the simulations [5], a number of other factors may lead to a substantial overestimation of the threshold fluences required for the onset of ablation and active molecular decomposition in the simulations. These factors include a higher density of the simulated targets, 1.55–1.83 g/cm³ versus 0.60–0.93 g/cm³ of the experimental samples, resulting in a stronger cohesion of the targets and smaller energy deposited per molecule at the same laser fluence and penetration depth. Moreover, the laser damage accumulation in the multi-pulse irradiation regime used in the experiments and a variety of alternative low-activation-energy bond breaking mechanisms that are not accounted for in the simulations may further reduce the experimental fluence thresholds. Nevertheless, at a qualitative level, the computational prediction on the existence of a window of fluences where the ejection and deposition of intact lysozyme molecules is possible can still be used in interpretation of the experimental observations.

The increase of target preparation pressure is likely to produce a more cohesive target capable of sustaining higher internal pressure. It means that the laser must supply more energy and, thereby, higher temperature and pressure in order to cause the collective material ejection. The higher temperature and pressure again lead to a significantly higher deposition rate measured by the weight enhancement on the substrate, i.e., the electrode of the QCM, Fig. 3. However, as seen from the MALDI measurements in Figs. 4 and 5, the number of intact molecules transferred to the substrate falls off by almost two orders of magnitude within the same pressure interval. The lysozyme molecules are presumably destroyed early in the ablation process by the high laser-induced pressure and temperature and not by the preparation pressure itself.

While there is an intuitive correlation between the laser fluence and maximum temperature reached in the target, the connection between the target compression pressure and temperature is less apparent. It can be expected, however, that the targets with increased pressure-induced cohesion of the lysozyme powder would require higher laser energy deposition and correspondingly higher values of temperature to trigger the ablation onset. Thus, while all targets are irradiated at the same incident laser fluence, it is reasonable to expect that the ablation starts earlier during the 6 ns laser irradiation for targets prepared at lower compressive pressure, and the cooling associated with material disintegration partially offsets the laser heating. As a result, the maximum values of temperature generated by the laser pulse can be reduced for the less cohesive targets down to the levels where the molecular decomposition is partially suppressed or completely avoided.

5. Conclusion

The preparation pressure of an organic target plays an important role in determining the film deposition rate and the relative number of intact molecules. We have investigated the variation of the deposition rate for the protein, lysozyme, with a QCM and quantitative MALDI. At an intermediate fluence of 2 J/cm², the deposition rate of the material (molecular fragments and/or intact molecules) rises from 3 to 9 ng/cm²

per shot within a pressure range from 10 bar to 160 bar. However, the number of intact molecules falls off by almost two orders of magnitude within the same pressure range. At a high compression pressure, the cohesion of the target powder is high, and a high temperature is needed for material ejection. This high temperature leads to an almost complete fragmentation of the lysozyme molecules in the ablation process.

The implication for the film deposition is that the films with most intact molecules should be produced with targets compressed at a pressure below 40 bar, but the pressure should also be high enough for preventing drop-out of chunks from the target holder, i.e., larger than about 40 bar for the lysozyme powder. A good compromise for functional films is thus a compression pressure in the range from 30 to 60 bar.

Acknowledgements

The authors want to thank Lotte Nielsen for the assistance with the MALDI measurements. LVZ acknowledges support by the National Science Foundation (NSF) through grant CMMI-1663429.

References

- [1] D.B. Chrisey, A. Pique, R.A. McGill, J.S. Horwitz, B.R. Ringeisen, D.M. Bubb, P.K. Wu, *Chem. Rev.* 103 (2003) 553.
- [2] G. Agarwal, R.S. Phadke, *Mater. Sci. Eng. C* 6 (1998) 13.
- [3] T. Smausz, G. Megyeri, R. Kékesi, C. Vass, E. György, F. Sima, I.O. Mihalescu, B. Hopp, *Thin Solid Films* 517 (2009) 4299.
- [4] E. György, A. Pérez del Pino, G. Sauthier, A. Figueras, *J. Appl. Phys.* 106 (2009) 114702.
- [5] M. Tabetah, A. Matei, C. Constantinescu, N.P. Mortensen, M. Dinescu, J. Schou, L.V. Zhigilei, *J. Phys. Chem. B* 118 (2014) 13290.
- [6] A. Purice, J. Schou, P. Kingshott, N. Pryds, M. Dinescu, *Appl. Surf. Sci.* 253 (2007) 6451.
- [7] A. Purice, J. Schou, N. Pryds, M. Filipescu, M. Dinescu, *Appl. Surf. Sci.* 254 (2007) 1244.
- [8] E. Pechkova, C. Nicolini, *J. Cryst. Growth* 231 (2001) 599.
- [9] E. Pechkova, C. Nicolini, *Nanotechnology* 13 (2002) 460.
- [10] B. Lösekrug, A. Meschede, H.-U. Krebs, *Appl. Phys. A Mater. Sci. Process.* 79 (2004) 1295.
- [11] E. Süske, *Appl. Surf. Sci.* 254 (2007) 1312.
- [12] G. Kecskeméti, B. Hopp, T. Smausz, Z. Tóth, G. Szabó, *Appl. Surf. Sci.* 258 (2012) 7982.
- [13] G. Kecskeméti, N. Kresz, T. Smausz, B. Hopp, A. Nógrádi, *Appl. Surf. Sci.* 247 (2005) 83.
- [14] L. Wang, D. Wen, *Sci. Rep.* 7 (2017) 17418.
- [15] P. Siebeneicher, H. Kleemann, K. Leo, B. Lüssem, *Appl. Phys. Lett.* 100 (2012) 193301.
- [16] Z. Hu, F. Huang and Y. Cao, *Small Methods*, 1, 1700264 (2017).
- [17] *Appl. Phys. A* (special issue on MAPLE) 105 (2011) 517–671.
- [18] Y. Guo, A. Morozov, D. Schneider, J.W. Chung, C. Zhang, M. Waldmann, N. Yao, G. Fytas, C.B. Arnold, R.D. Priestley, *Nat. Mater.* 11 (2011) 337.
- [19] A. Purice, J. Schou, P. Kingshott, M. Dinescu, *Chem. Phys. Lett.* 435 (2007) 350.
- [20] S. Canulescu, J. Schou, S.F. Nielsen, K.V. Hansen, H. Conseil, *Chem. Phys. Lett.* 588 (2013) 119.
- [21] E. Leveugle, L.V. Zhigilei, *J. Appl. Phys.* 102 (2007) 074914.
- [22] E. Leveugle, A. Sellinger, J.M. Fitz-Gerald, L.V. Zhigilei, *Phys. Rev. Lett.* 98 (2007) 216101.
- [23] A. Matei, J. Schou, C. Constantinescu, P. Kingshott, M. Dinescu, *Appl. Phys. A Mater. Sci. Process.* 105 (2011) 629.
- [24] C. Constantinescu, A. Matei, J. Schou, S. Canulescu, M. Dinescu, *Appl. Phys. B Lasers Opt.* 113 (2013) 367.
- [25] S. Severin, X. Wenshui, *Crit. Rev. Food Sci. Nutr.* 45 (2005) 645.
- [26] P. Kingshott, H. Thissen, H.J. Griesser, *J. Biomed. Mater. Res.* 49 (2000) 36.
- [27] W. Svendsen, J. Schou, T.N. Hansen, O. Ellegaard, *Appl. Phys. A* (66) (1998) 493.
- [28] T. Smausz, B. Hopp, H. Huszár, Z. Tóth, G. Kecskeméti, *Appl. Phys. A Mater. Sci. Process.* 79 (2004) 1101.
- [29] C.E. Kundrot, F.M. Richards, *J. Mol. Biol.* 200 (1988) 401.
- [30] H. Durchschlag, T. Hefferle, P. Zipper, *Radiat. Phys. Chem.* 67 (2003) 479.