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

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.
The minimum amount of "matrix " needed for matrix-assisted pulsed laser deposition of biomolecules

The ability of matrix-assisted pulsed laser evaporation (MAPLE) technique to transfer and deposit high-quality thin organic, bioorganic, and composite films with minimum chemical modification of the target material has been utilized in numerous applications. One of the outstanding problems in MAPLE film deposition, however, is the presence of residual solvent (matrix) codeposited with the polymer material and adversely affecting the quality of the deposited films. In this work, we investigate the possibility of alleviating this problem by reducing the amount of matrix in the target. A series of coarse-grained molecular dynamics simulations are performed for a model lysozyme-water system, where the water serves the role of volatile "matrix" that drives the ejection of the biomolecules. The simulations reveal a remarkable ability of a small (5-10 wt %) amount of matrix to cause the ejection of intact bioorganic molecules. The results obtained for different laser fluences and water concentrations are used to establish a "processing map" of the regimes of molecular ejection in matrix-assisted pulsed laser deposition. The computational predictions are supported by the experimental observation of the ejection of intact lysozyme molecules from pressed lysozyme targets containing small amounts of residual water. The results of this study suggest a new approach for deposition of thin films of bioorganic molecules with minimum chemical modification of the molecular structure and minimum involvement of solvent into the deposition process. (Graph Presented).

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Laser ablation of lysozyme with UV, visible and infrared femto- and nanosecond pulses
Lysozyme is an interesting molecule for laser ablation of organic materials, because the ablation has been comprehensively studied, it is a medium heavy molecule with a mass of 14305 Da, which can be detected by standard techniques, and because it is used as a bactericidal protein in the food industry. Lysozyme molecules do not absorb energy for wavelengths above 310 nm, but nevertheless there is a strong mass loss by ablation for laser irradiation in the visible regime. The total ablation yield of lysozyme at 355 nm and at 2 J/cm2 is about 155 µg/pulse, possibly one of the highest ablation yields ever measured. The mass loss is mainly caused by fragmentation of the lysozyme into simple gases, such as H2S, H2O and CO2, which are rapidly pumped away in the vacuum chamber.

We have investigated the mass loss by ablation of lysozyme in all regimes to see whether a similar mechanism governs the ablation process for different wavelengths and time duration. Measurements for 6-7-ns laser ablation were carried out.
A study on matrix assisted pulsed evaporation (MAPLE) of organic materials

Organic films can be produced either by MAPLE or directly by PLD (Pulsed laser deposition). For a reasonable deposition rate of ng/cm² per pulse for film production by MAPLE a fluence of 1-1.5 J/cm² is required at the laser wavelength of 355 nm, while the fluence can be considerably lower at 248 nm. At high fluence the deposition rate of proteins by MAPLE seems to decrease. The surface roughness is still an issue, but at low fluence it seems to be acceptable. The fragmentation rate increases with fluence, and seems to be less pronounced for MAPLE than for PLD. Also this issue is not yet resolved.

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Laser ablation of the protein lysozyme

Lysozyme is a well-known protein, which is used in food processing because of its bactericidal properties. The mass (14307 amu) is in the range in which it easily can be monitored by mass spectrometric methods, for example by MALDI (Matrix Assisted Laser Desorption ionization). We have recently produced thin films of average thickness up to 300 nm, which not only contained a significant amount of intact molecules, but also maintained the bioactivity. These films were produced by a nanosecond laser in the UV regime at 355 nm with 2 J/cm². The surprising fact that these molecules can be transferred to a substrate as intact molecules by the violent laser impact (up to 50 mJ/pulse) has not yet been understood. One issue is that up to 150 ng/pulse is removed by the laser, and much of the material is ejected from the target in relatively large chunks. We have explored as well the excitation mechanics by laser impact. Samples of pressed lysozyme prepared in the same manner as in ns-experiments have been irradiated at 527 nm with >>300-fs pulses and at a similar fluence as in ns ablation. Even though the pulse energy was much smaller, there was a considerable ablation weight loss of lysozyme from each shot. This is the first time the ablation by fs-lasers of a protein has been recorded quantitatively. Films of lysozyme produced by fs-laser irradiation were analyzed by MALDI and a significant number of intact
Pulsed laser deposition of the lysozyme protein: an unexpected "Inverse MAPLE" process

Films of organic materials are commonly deposited by laser assisted methods, such as MAPLE (matrix-assisted pulsed laser evaporation), where a few percent of the film material in the target is protected by a light-absorbing volatile matrix. Another possibility is to irradiate the dry organic material directly for film production, as in PLD (pulsed laser deposition), where the film molecules may undergo strong fragmentation. In this presentation we report an alternative surprising mechanism for film deposition of the protein lysozyme in vacuum, when a small amount of residual water drives the ejection and deposition of lysozyme. This can be called an “inverse MAPLE” process, since the ratio of “matrix” to film material in the target is 10:90, which is inverse of the typical MAPLE process where the film material is dissolved in the matrix down to several wt. %.

Lysozyme is a well-known protein which is used in food processing and is also an important constituent of human secretions such as sweat and saliva. It has a well-defined mass (14307 u) and can easily be detected by mass spectrometric methods such as MALDI (Matrix-assisted laser desorption ionization) in contrast to many other organic materials. Also, the thermal properties of lysozyme, including the heat-induced decomposition behavior are comparatively well-known.

The ablation of lysozyme from a dry pressed target in vacuum was measured by weight loss in nanosecond laser ablation at 355 with a fluence of 0.5 to 6 J/cm². Films with a significant number of intact lysozyme molecules have been produced by direct laser irradiation of a pressed target and the number of intact molecules shows a maximum at around 2.5 J/cm². Apparently, there is a certain range of laser fluences when the transfer of intact lysozyme to the film substrate is possible. The experimental results are explained with the help of molecular-level computer simulations. The simulations show that pure lysozyme cannot ablate without complete fragmentation. However, small pockets of trapped water provide the necessary expansion of the target and the ejection of intact lysozyme molecules above a certain fluence threshold, below which no lysozyme molecules are ejected. For high fluences all molecules are ejected as fragments. For a reasonable concentration of water (10%) the fluence dependence similar to that obtained experimentally is observed in the simulations.

Laser ablation dynamics and production of thin films of lysozyme

Lysozyme is a well-known protein, which is used in food processing because of its bacteriocidal properties. The mass (14307 u) is in the range, in which it easily can be controlled by mass spectrometric methods, for example by MALDI (Matrix assisted laser desorption ionisation). We have recently at the Technical University of Denmark (DTU) produced thin films of average thickness up to 300 nm, which not only contained a significant amount of intact molecules, but also maintained the bioactivity. These films were produced by a nanosecond laser in the UV regime at 355 nm with 2 J/cm². The surprising fact that these molecules can be transferred to a substrate as intact molecules by the violent laser impact (~up to 50 mJ/pulse) has not yet been understood. One issue is that up to 150 ng/pulse is removed by the laser, and much of the material is ejected from the target in relatively large chunks. We have continued these experiments at CNR-SPIN, Napoli, to explore the excitation mechanics by laser impact. Samples of pressed lysozyme prepared in the same manner
as in DTU have been irradiated at 523 nm with 300-fs pulses and a fluence of the same order of magnitude as in DYU. Even though the pulse energy was much smaller, there was a considerable ablation weight loss of lysozyme from each shot. This is the first time the ablation by fs-lasers of a protein has been recorded quantitatively. Films of lysozyme produced by fs-laser irradiation will be analysed by MALDI in order to explore if there also is a significant amount of intact molecules in the films for fs-laser deposition.

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**Laser ablation of the lysozyme protein: a model system for soft materials**
Lysozyme is a well-known protein which is used in food processing and is also an important constituent of human secretions such as sweat and saliva. It has a well-defined mass (14307 u) and can easily be detected by mass spectrometric methods such as MALDI (Matrix-assisted laser desorption ionization) in contrast to many other organic materials. Also the thermal properties, including the heat-induced decomposition behavior are comparatively well-known. For laser-irradiation at wavelengths above 310 nm, no photochemical processes occur initially, but the material is ejected via photothermal processes. The ablation of lysozyme from a dry pressed target in vacuum was measured by weight loss for nanosecond and femtosecond laser pulses at 355 or around 532 nm with a fluence of 1 J/cm². A typical ablation yield for a 10-mJ pulse is about 150 micrograms/pulse, corresponding to the removal of ~ 6.3 10¹⁵ molecules per pulse. This is perhaps one of the highest ablation yields ever measured. Films with a significant number of intact lysozyme molecules have been produced by PLD (pulsed laser deposition) and MAPLE (Matrix-assisted pulsed laser evaporation). The deposition of intact molecules is expected in MAPLE, but is surprising in PLD, where a high degree of thermal fragmentation is typically required for generation of a sufficient amount of volatile decomposition products that drive the transfer of molecules to the film substrate. The experimental results will be discussed based on the results of molecular-level modeling. In particular, the effect of the possible presence of trapped water pockets in the lysozyme targets is investigated in the simulations and the minimum amount of water required for the lift off of the intact molecules is established.

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