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Laser Assisted Crystallization: An alternative tool to crystallize biomolecules

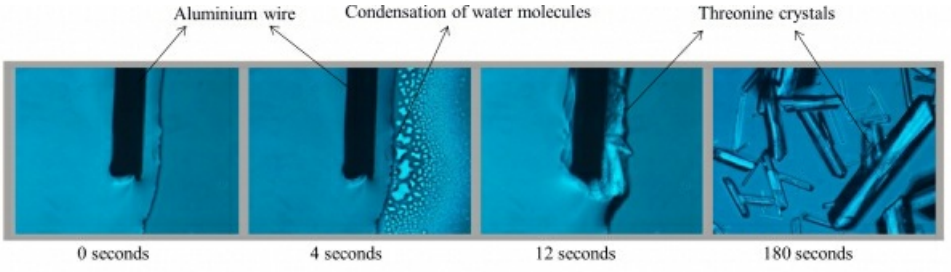
Abdul Ajees Abdul Salam[1], Shilpa T[2], Sajan D. George[2], Aseefhali Bankapur[2], Santhosh Chidangil[2], Aditya K. Dharmadhikari[3], Deepak K Mathur[3]

[1]*Department Of Atomic And Molecular Physics, Manipal University, Manipal, India,* [2]*Manipal University, Manipal, India,* [3]*Tata Institute of Fundamental Research, Mumbai, India*

E-mail: abdul.ajees@manipal.edu

Knowledge of 3D structures is a prerequisite for developing structure-based drug design and discovery of novel drugs and pharmaceutical products. The human genome contains protein-coding genes in between 20,000 to 25,000 and many proteins playing a key role in the living mechanisms have not been crystallized so far. Developing high throughput, automatic and rapid protein crystallization method is critical, and laser-induced crystallization is one of the promising alternatives in this regard. We have used an ultrafast laser (800 nm wavelength, 60 fs pulse duration, 5.2 MHz repetition rate, 300 mW average power) to crystallize NaCl, five chalcone compounds, and a lysozyme protein [1]. Recently, we conducted a systematic study in which we successfully crystallized biomolecules using a continuous wave Nd:YAG (wavelength = 1064 nm) laser using low cost and readily available absorbing nucleants like copper wire, aluminum wire, copper particles, aluminum particles, graphite particles, as well as multi-walled carbon nanotubes (MCWNT). We illustrate the potential of our approach by first crystallizing standard small molecules - NaCl and KCl – and glycine in solution form under varying conditions of laser power and irradiation time. Optimized values of these parameters are then utilized to crystallize 20 amino acids and lysozyme protein. The prepared crystals were further characterized using single crystal X-ray diffraction and Raman spectroscopy. The current study elucidates that the femtosecond laser based crystallization offers advantages such as use of very low laser powers (~4 mW/cm^2), extremely rapid crystallization (~3 seconds), use of biomolecules in low concentration (0.5 M), etc. Though high-throughput technologies have evolved for crystallization of macromolecules, such crystallization still requires time periods ranging from several hours to weeks to confirm whether or not nucleation has occurred. Our technique might offer new avenues to understand the nucleation mechanism and to have better control over the crystallization process. This kind of approach may facilitate the development of protocols that enable preparation of diffraction quality crystals of small molecules, and of seeding crystals for peptides and proteins on significantly shorter timescales than hitherto possible. In our laboratory, we are exploring this technique to obtain the crystals of protein molecules.

[1] T. Shilpa et al. [2015], Proc. Indian Natl. Sci. Acad., 81, 517-523.



Representative of time evolution of L-threonine crystals upon the irradiation of 1064nm laser on the tip of the aluminium wire

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