Acta Crystallographica Section F
Structural Biology and Crystallization Communications
ISSN 1744-3091

Laser-improved protein crystallization screening

Neela Yennawar,[a]* Sava Denev,[b] Venkataraman Gopalan[b] and Hemant Yennawar[c]

[a]Huck Institutes of Life Sciences, Pennsylvania State University, University Park, PA 16802, USA, [b]Materials Research Institute, The Pennsylvania State University, University Park, PA 16802, USA, and [c]Department of Biochemistry and Molecular Biology, The Pennsylvania State University, University Park, PA 16802, USA

Correspondence e-mail: nhy1@psu.edu

Received 21 April 2010
Accepted 19 June 2010

Screening of proteins for crystallization under laser irradiation was investigated using six proteins: ribonuclease B, glucose dehydrogenase, lysozyme, sorbitol dehydrogenase, fructose dehydrogenase and myoglobin. Shining 532 nm green circularly polarized laser light with a picosecond pulse and 6 mW power for 30 s on newly set-up protein drops showed a marked improvement in the number of screen conditions amenable for crystal growth compared with control drops under identical conditions but without laser exposure. For glucose dehydrogenase and sorbitol dehydrogenase, larger and better quality crystals were formed and the resolution of X-ray diffraction was improved. The speed of crystallization increased in the case of ribonuclease B, lysozyme and sorbitol dehydrogenase. During laser irradiation, the amount of precipitation in the screened drops increased, indicating a transient decrease in protein solubility. At the optimized laser settings, there was no deleterious effect of the laser on crystal growth or on the protein. In the cases of ribonuclease B and lysozyme the crystal packing did not change owing to the laser exposure.

1. Introduction

The crystallization of biological macromolecules is usually the rate-limiting step in crystal structure determination. Typically, hundreds of conditions are tried in the hope of obtaining a few promising leads. These few hits are then adjusted in a multi-grid experiment to optimize the concentration, pH and various additives in order to obtain crystals of good quality and size; overall, a chancy and time-consuming endeavor. Any technique that could improve the probability of obtaining crystals as well as expedite the crystallization process is of great value. Earlier studies on lysozyme have shown that light irradiation of 30 mg ml^{-1} lysozyme solutions for 120 s using a Xe lamp produces white turbidity in solutions containing NaCl. The process has been described as photochemically induced nucleation (Veesler *et al.*, 2006). The first report of nonphotochemical laser-induced nucleation (NPLIN) of protein crystals was by Adachi *et al.* (2003), who used femtosecond laser pulses to induce a nonlinear photophysical nucleation. This is different from the NPLIN method of Lee *et al.* (2008), in which polarized nanosecond or picosecond laser pulses were used to induce an electric field-dependent reorganization of lysozyme molecules into a crystalline state. We have explored the effects of NPLIN using a picosecond laser on six proteins: ribonuclease B, sheep liver sorbitol dehydrogenase, glucose dehydrogenase, lysozyme, fructose dehydrogenase and myoglobin. We looked for changes in any of the following on laser irradiation: (i) hits for crystallization conditions, (ii) crystal size, (iii) resolution of diffraction data, (iv) speed of crystal growth and (v) crystal packing.

The laser used for the purpose was 532 nm green circularly polarized light with a picosecond pulse. Initial runs with circular *versus* linear polarized light showed no difference in results. The diameter of the light beam was adjusted to that of the crystallization

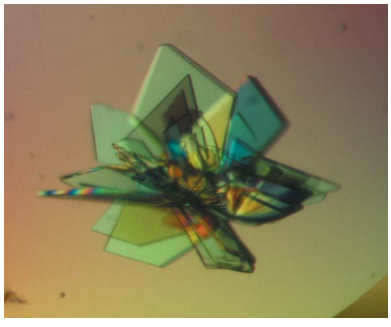


Table 1
Comparison of laser crystallization with control.

		Laser		Control		Maximum resolution at home source (Å)	
Protein	Screen tried	Conditions that nucleated	Time taken	Conditions that nucleated	Time taken	Laser	Control
Lysozyme	Index 1–48	22, 33, 34, 38	3–4 h	33, 34, 38	48 h	1.7	1.7
Ribonuclease B	Index 49–96	81	1 d	81	3 weeks	1.85	1.85
Sorbitol dehydrogenase	PEG/Ion 1–48	2, 3, 14, 21, 22, 23, 24, 25, 27, 30, 37, 38, 40, 44	48 h	2, 3, 20, 21, 22, 23, 36, 37, 40	1 week	1.75	3.0
Glucose dehydrogenase	PEG/Ion 1–48	1, 5, 7, 9, 20, 21, 48	1 week	1, 7, 9, 21, 24, 48	1 week	2.0	No diffraction
Myoglobin	Index	95	1 d	95	1 week	No diffraction	No diffraction
Fructose dehydrogenase	PEG/Ion	30	3 weeks	No crystals	No crystals	No diffraction	No crystals

drop. Varying exposure times (30, 60 and 90 s) and laser power (6, 9 and 12 mW) were initially tested for lysozyme. High exposure times or power resulted in poor and damaged crystals. The optimum condition was found to be 30 s exposure at 6 mW power. These laser settings were used for the rest of the samples. The drops were incubated for 30 min after setup and before laser irradiation. Hampton Research screens (http://www.hamptonresearch.com) were used for the crystallization of each protein sample. All protein samples were purchased from Sigma (http://www.sigmaaldrich.com). The sitting-drop vapor-diffusion method was employed with a Hampton Research Cryschem plate; drops consisted of 1 µl protein solution and 1 µl reservoir solution and the reservoir contained 500 µl screen solution. One set of drops was subjected to laser exposure and the other, a control set, was not. Both sets of drops were kept under identical conditions and monitored simultaneously over the next few days. The results in each case are reported in the following (Fig. 1, Table 1).

2. Experimental and results

2.1. Ribonuclease B

Previous studies have shown that ribonuclease B crystals take about three weeks to grow (Williams *et al.*, 1987). In our study, Hampton Research Index conditions 49–96 were set up with 20 mg ml^{-1} ribonuclease B solution in water. The drops exposed to laser radiation yielded good-quality crystals of 200 µm in size in Index condition No. 81 in 1 d. One of four control drops with the same screen condition yielded crystals in three weeks. For cryo-solvent we used 20 µl Index condition No. 81 and 5 µl 50% PEG 4K. A 150 µm plate crystal was soaked in the cryo-buffer for 15 min before freezing in a nitrogen stream. Three-dimensional X-ray diffraction data were collected to 1.85 Å resolution using our in-house MicroMax-007 HF generator and a Saturn 944+ CCD detector. These crystals belonged to the monoclinic space group $P2_1$, with unit-cell parameters $a = 40.2$, $b = 58.6$, $c = 54.5$ Å, $\beta = 96.8^\circ$. The control-drop crystals were confirmed to have the same unit cell.

2.2. Sorbitol dehydrogenase from sheep liver

The crystallization of sheep liver sorbitol dehydrogenase has not been reported to date. Hampton Research PEG/Ion Screen conditions 1–48 was used for crystal screening. Using the laser, 17 of the 48 conditions produced crystals. Three of the 17 conditions (Nos. 24, 30 and 40) produced crystals of suitable size and quality for the collection of three-dimensional X-ray diffraction data. The laser-induced crystals from condition No. 24 diffracted to 1.75 Å resolution. Without the use of the laser, nine of the 48 conditions yielded thin needles. The best needle crystals were from PEG/Ion Screen condition No. 24. Repeating PEG/Ion Screen condition No. 24 with four control drops led to one drop that contained a crystal that diffracted to about 3.0 Å resolution (after a cryo-annealing procedure). The remaining three control drops were clear, indicating difficulty in nucleation. The unit-cell parameters of the laser-induced and control crystals were similar, indicating no change in crystal packing.

2.3. Glucose dehydrogenase from *Bacillus megaterium*

In the literature, crystals of this enzyme were grown in a two-step procedure (Yamamoto *et al.*, 2000). In the first step small crystals were grown in two weeks which were used in the second step as seeds. Larger crystals grew in another two weeks. In our trials, sitting-drop crystallization trays were set up using Hampton Research PEG/Ion Screen. In a single step, laser-irradiated drops corresponding to condition Nos. 1, 5, 7, 9, 20, 21 and 48 of the screen produced crystals, of which condition Nos. 7, 9 and 48 led to crystals of about 160–200 µm in size in one week. We were successful in collecting a 2.0 Å resolution data set from the laser-grown crystals. The unit-cell parameters and space group of these crystals ($a = 55.7$, $b = 67.8$, $c = 132.1$ Å, $\alpha = 82.1$, $\beta = 93.2$, $\gamma = 74.5^\circ$, space group $P1$) were different compared with those published previously ($a = 120.8$, $b = 66.7$, $c = 119.6$ Å, $\beta = 93.25^\circ$, space group $C2$; Yamamoto *et al.*, 2000). The crystals from the control grew in Hampton Research PEG/Ion Screen condition Nos. 1, 7, 9, 21, 24 and 48 and were too small to handle for diffraction testing. The best crystals from the control drops grew in condition No. 48 as 50 µm two-dimensional thin plates that were too small for diffraction testing.

2.4. Lysozyme

Sitting drops were set up with Hampton Index screen conditions 1–48 using lysozyme samples at 30, 45 and 60 mg ml^{-1}. Several crystals grew in Index condition Nos. 22, 33, 34 and 38 in 3–4 h in the laser-irradiated drops, while the control drops remained clear. The higher the concentration, the faster the crystal growth. In comparison, crystals grew in the control drops in 2 d in Index condition Nos. 33, 34 and 38. A new condition (Index No. 22) was identified for which the control drops contained no crystals while the laser-irradiated drops contained crystals. X-ray screening of all of the conditions confirmed that we had grown protein crystals with unit-cell parameters close to those of the tetragonal lysozyme cell. Hence, the laser had not changed the packing and had assisted in speeding up crystallization and nucleation in a new crystallization condition.

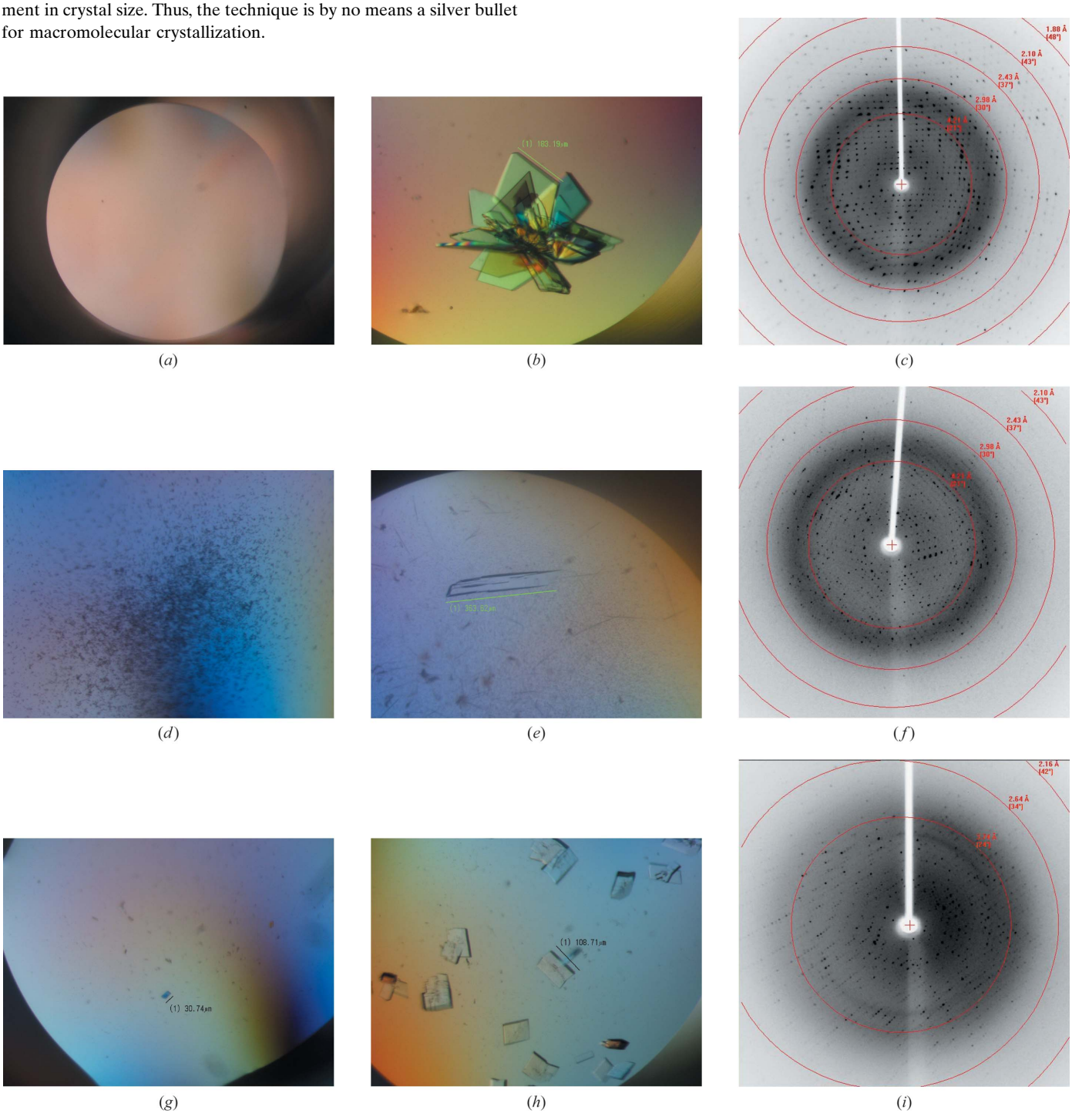
2.5. Other examples

Fructose dehydrogenase from *Gluconobacter* sp. has not been reported to form crystals in the available literature. Using the laser technique, we observed very thin needles about 350 µm in length that

grew in about three weeks in condition No. 30 of PEG/Ion screen. No crystals were seen in the control drops. However, these crystals were too fragile to be tested for diffraction. In the case of myoglobin, poor-quality plate-like crystals formed both with and without the laser in Hampton Index condition No. 95. These grew more rapidly with the laser (1 d) compared with the control (one week), but neither showed any diffraction. We also tried an RNA provided by the Bevilacqua laboratory (Pennsylvania State University) and found no improvement in crystal size. Thus, the technique is by no means a silver bullet for macromolecular crystallization.

3. Conclusions

Protein crystallization is the rate-limiting step in many structural biology projects. Methods to improve protein crystal growth are useful to ensure greater success in understanding the three-dimensional structure of proteins. We have explored the technique of NPLIN as a viable tool to screen proteins for crystal growth and seen significant improvement in crystal size, quality, growth speed, reso-



(*a*) (*b*) (*c*)

(*d*) (*e*) (*f*)

(*g*) (*h*) (*i*)

Figure 1
Laser-improved crystals of (*b*) ribonuclease B grown in 1 d, (*e*) sorbitol dehydrogenase grown in 2 d and (*h*) glucose dehydrogenase grown in one week with laser irradiation. Corresponding diffraction patterns are shown in (*c*), (*f*) and (*i*), respectively. At the time points where these crystals were observed, equivalent control drops are shown in (*a*), (*d*) and (*g*), respectively.

lution of diffraction and number of positive hits during screening of five proteins: hen egg-white lysozyme, bovine pancreas ribonuclease B, sheep liver sorbitol dehydrogenase, *Gluconobacter* fructose dehydrogenase and *B. megaterium* glucose dehydrogenase. Further studies are ongoing to test the utility of the laser to crystallize other macromolecules and to understand how the laser helps in increasing nucleation.

We would like to acknowledge the NIH–NCRR shared instrumentation grant 1S10RR023439-01 and the PSU centre for optical technologies.

References

Adachi, H., Takano, K., Hosokawa, Y., Inoue, T., Mori, Y., Matsumura, H., Yoshimura, M., Tsunaka, Y., Morikawa, M., Kanaya, S., Masuhara, H., Kai, Y. & Sasaki, T. (2003). *Jpn J. Appl. Phys.* **42**, 798–800.

Lee, I. S., Evans, J. M. B., Erdemir, D., Lee, A. Y., Garetz, B. A. & Myerson, A. S. (2008). *Cryst. Growth Des.* **8**, 4255–4261.

Veesler, S., Furuta, K., Horiuchi, H., Hiratsuka, H., Ferte, N. & Okutsu, T. (2006). *Cryst. Growth Des.* **6**, 1631–1635.

Williams, R. L., Greene, A. M. & McPherson, A. (1987). *J. Biol. Chem.* **262**, 16020–16031.

Yamamoto, K., Kusunoki, M., Urabe, I., Tabata, S. & Osaki, S. (2000). *Acta Cryst.* D**56**, 1443–1445.