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LASER-INDUCED NUCLEATION OF VIRUSES

An Original Proposal by Joseph Talafous

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One central unifying idea in chemistry is that structure and properties are related. Similarly, the functional diversity of the life processes is rooted in the chemical structures of molecular biology. Viruses provide a very elegant proof of this. On one hand, a virus is definitely a chemical. Some viruses can be purified to homogeneity, and remain on the shelf for years. Viruses can be treated like any familiar chemical - analyzed, derivatized, weighed, etc. The atomic structure can be known to near perfect detail. But on the other hand, this chemical can exhibit "non-chemical" behavior. It can enter a cell at will, insert itself into the cell's genome, stay there for many years, but when the cell is in trouble, the virus forces the cell to massively reproduce the viral genome and synthesize the protein packing coat for the virus too. Then in a most basic chemical sense, the protein packing material and the viral genome self-assemble chemically by themselves, and then exit the cell, incorporating into itself any of the cell's membrane it may need.

This fascinating "chemical" is also very deadly. Our lack of detailed chemical knowledge about it makes us defenseless to design chemical weapons. Our chemical knowledge increases greatly when we get the detailed atomic structure of the virus. The only successful way to get the coordinates of such large structures is through X-ray crystallography. Unfortunately, the bottleneck in the process of X-ray crystallography is the procurement of high-quality crystals. Viruses are especially difficult to crystallize. Tremendous effort goes into crystallizing proteins such as growth on the space shuttle[1] or building expensive robots to automatically search parameter space.[2] Many Ph.D. theses describe searching parameter space for crystallization of a single protein. It is a big problem, the crystallographer McPherson says: "Failure to obtain crystals ... hinders basic understanding of natural macromolecular interactions" and "there are only a few empirical approaches [to crystallization]".[3]

The difficulty in the crystallization of viruses has not stopped virologists from routinely building very low-resolution structures of viruses though image reconstruction involving averaging electron microscopy images.[4] Recently, however, the crystallographer Rossmann succeeded in crystallizing and solving the structure of the rhino virus.[5] Much has been learned as a result.

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[1]Littke, W.; John, C. Protein Single Crystal Growth under Microgravity, *Science*, **225**, 203-204 (1984); DeLucas, L.J.; Smith, C.D.; Smith, W. Vijay-Kumar, S.; Senadhi, S.E.; Ealick, S.E.; Carter, D.C.; Snyder, R.S.; Weber, P.C.; Salemme, F.R.; Ohlendorf, D.H.; Einspahr, H.M.; Clancy, L.L.; Navia, M.A.; McKeever, B.M.; Nagabhushan, T.L.; Nelson, G.; McPherson, A.; Koszelak, S.; Taylor, G.; Stammers, D.; Powell, K.; Darby, G.; Bugg, C.E.; Protein Crystal Growth in Microgravity, *Science* **246**, 651-654 (1989).

[2]Cox, M.J.,;Weber, P.C. Experiments with Automated Protein Crystallizaton. *J. Appl. Crystallog.*, **20**, 366 (1988).; Kelders, H.A., Kalk, K.H., Gros, P., Hol., W. *Protein Engineering*, **1**, 301, (1987).

[3]McPherson, A. Macromolecular Crystals, *Sci. Amer.* 62-69, (March 1989).

[4]Stewart, P.L.; Burnett, R.M.; Cyrklaff, M.; Fuller, S.D. Image Reconstruction Reveals the Complex Molecular Organization of Adenovirus, *Cell*, **67**, 145-154, (Oct. 1991).

[5]Arnold, E.; Rossmann, M.G.; Analysis of the Structure of a Common Cold Virus, Human Rhinovirus 14, Refined at a Resolution of 3.0 A, *J. Mol. Biol.*, **211**, 763-801 (1990).

For example, the binding of an antiviral drug was elucidated.[6] This type of information may speed up the cure for the common cold.

To make matters more complicated, lack of nucleation can prevent crystal growth. It is entirely possible that crystallization conditions that are not observed to yield crystal really would, if a seed crystal were present. In practice or principle, the extent that non-nucleation deters crystallization is not well understood. It might actually be that most conditions would allow viral crystal growth, but nucleation of the viruses does not occur and prevents it. This project will discuss a potential new approach for the nucleation of viruses.

The proposed method will employ laser pressure to hold a few unit cells of virus together, which is enough to form a crystal nucleus.[7] Feasibility of my strategy is demonstrated by a long line of research originated by Ashkin[8] who noticed that laser light which is focused to nearly one wavelength can be used to influence the motion of micron-sized latex spheres. This influence is not slight; the acceleration on the spheres can be as large as one million g.[9] The forces could not be explained as photophoretic or radiometric in origin as had been observed in previous studies.[10] The latex spheres were attracted to the axis of the laser beam and simultaneously along it, away from the laser, trapping it on the opposing surface of its container. Ashkin experimented and found that the index of refraction is a major factor, and constructed an explanation of the effect based on classical optics.

Subsequently, a vertical laser beam was used to levitate small transparent glass beads trapped in a perpendicular horizontal beam of equal intensity.[11] The transverse forces (attracting the sphere to the laser axis) were stronger, as evidenced by the inability for the vertical beam to push the sphere out of the horizontal beam. Trapping between the focal points of opposing lasers was also shown to be possible. The potential energy depth of the optical well was adjustable (even to a depth many times higher than thermal energy) by adjusting the intensities. Another study found that liquid oil drops in the size range of 1-40 microns could be easily manipulated and coalesced by "optical tweezers."[12] However, it was found that the trapping does not work in a complete vacuum due to underdamped laser fluctuations.[13]

Laser trapping has been applied to many new systems ranging from the atomic level to barely visible by the naked eye. For example, properties of isolated atoms have been determined using laser trapping of single atoms[14] and this avenue of research has been very fertile. Atom states

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[6]Chapman, M.S.; Minor, I.; Rossmann, M.G. Human Rhinovirus 14 Complexed with Antiviral Compound R 61837, *J.Mol. Biol.*, **217**, 455-463, (1991).

[7]Feher, G. Mechanisms of Nucleation and Growth of Protein Crystals, *Journal of Crystal Growth*, **76**, 545-546 (1986).

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[8]Ashkin, A., Acceleration and Trapping of Particles by Radiation Pressure, *Phys. Rev. Lett.*, **24**, 156 (1970).

[9]Ashkin, A., The Pressure of Laser Light, *Sci. Amer.*, 63-71 (February 1972).

[10]May, A.D., Rawson, E.G., Hara, E.H., *J. Appl. Phys.*, **38**, 5290 (1967).

[11]Ashkin, A.; Dziedic, J.M. Optical Levitation by Radiation Pressure, *Appl. Phys. Lett.*, **19**, 283-285, (1971).

[12]Ashkin, A.; Dziedzic, J.M. Optical Levitation of Liquid Drops by Radiation Pressure, *Science*, **187**, 1073-1075, (1975).

[13]Ashkin, A.; Dziedzic, J.M. Optical levitation in high vacuum, *Appl. Phys. Lett.*, **28**, 333-335, (1976).

[14]Raab, E.L.; Prentiss, M.; Cable, A.; Chu, S.; Pritchard, D.E.; Trapping of Neutral Sodium Atoms with Radiation Pressure, *Phys. Rev. Lett.*, **59**, 2631-2634 (1987).; Pritchard, D.E.; Raab, E.L.; Bagnato, V.; Wieman, C.E.;

are characterizable in such systems. Ashkin realized[15] the practical uses of manipulation of micron-sized particles, among them being shape determination of single particles using Mie light-scattering,[16] highly accurate spectroscopic measurements,[17] and holding thermonuclear fusion targets.[18]

Laser trapping has also been applied successfully to microorganisms. Single yeast cells have been trapped and dragged at 100 micron per second using 80 mW IR laser beam; the yeast cells reproduced while in the trap, which indicated no major damage occurred.[19] The irregularly shaped *E. coli* bacterium and the highly elongated TMV virus[20] have also been trapped. Cellular organelles such as chromosomes and chloroplasts have been moved within living cells without death of the cell.[21]

With the appropriate experimental set-up, one-dimensional self-organization of the spheres can be accomplished from long-range forces caused by the light in what has been called "optical binding."[22] This overall effect is caused by the light scattered from one sphere influencing another sphere; the first sphere sets up a second trap for the second sphere.[23]

Laser radiation pressure can also impose two-dimensional order. In one study, 2D arrays of 0.2 micron polystyrene spheres have been induced by a periodic trap, as evidenced by Bragg reflections from a third laser.[24] Furthermore, the hexagonal closest packed arrays that resulted may have provided a nucleus to form cubic closest packed crystals, but this is not certain. This effect has been used to study phase transitions in colloidal solutions because this effect resembles freezing.[25] Tight control over the 2D crystal packing was recently achieved using diffraction gratings that yield any two-dimensional pattern.[26]

Watts, R.N.; Light Traps Using Spontaneous Forces, *Phys. Rev. Lett*, **57**, 310-313 (1986).; Bagnato, V.S.; Lafyatis, G.P.; Martin, A.G.; Raab, E.L.; Ahmad-Bitar, R.N.; Pritchard, D.E. Continuous Stopping and Trapping of Neutral Atoms. *Phys. Rev. Lett.*, **58**, 2194-2197 (1987).

[15]Ashkin, A. Applications of Laser Radiation Pressure, *Science*, **210**, 1081-1088 (1980).

[16]; Guillame, B. Delfour, A.; Bize, D. 10.6μm Mie scattering by a single particle in optical levitation. *Proc. Soc. Photo-Opt. Eng.*, **384**, 66-72 (1983).

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[18]Ashkin, A.; Dziedzic, J.M. *Appl. Phys. Lett.*, **30**, 202 (1977).

[19]Ashkin, A.; Dziedzic, J.M; Yamane, T. Optical trapping and manipulation of single cells using infared laser beams., *Nature*, **330**, 769-771 (1987).

[20]Ashkin, A.; Dziedzic, J.M. Optical Trapping and Manipulation of Viruses and Bacteria, *Science*, **235**, 1517-1520, (1987).

[21]Block, S.M.; Blair, D.F.; Berg, H.C. Compliance of bacterial flagella measured with optical tweezers, *Nature*, **338**, 514-518, (1989).; Ashkin, A.; Dziedzic, J.M. Internal cell manipulation using infared laser traps, *Proc. Natl. Acad. Sci. USA*, **86**, 7914-7918, (1989).; Berns, M. W.; Wright, W.H.; Tromberg, B.J.; Profeta, G.A.; Andrews, J.J.; Walter, R.J. Use of a laser-induced optical force trap to study chromosome movement on the mitotic spindle, *Proc. Natl. Acad. Sci.* USA, **86**, 4539-4543, (1989).

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[23]Smith, P.W.; Ashkin, A.; Tomlinson, W.J. *Opt. Lett.*, **6**, 284 (1981).

[24]Clark, N.A.; Hurd, A.J.; Ackerson, B.J. Single colloidal crystals, *Nature*, **281**, 57-60 (1979).

[25]Chowdhury, A.; Ackerson, B.J. Laser-Induced Freezing, *Phys. Rev. Lett.*, **55**, 833-836 (1985).

[26]Burns, M.M.; Fournier, J-M; Golovchenko, J.A. Optical Matter: Crystallization and Binding in Intense Optical Fields, *Science*, **249**, 749-754.

I propose that a three-dimensional periodic laser trap be constructed that provides a template for the nucleus to form. This novel technique will induce 3D crystalline order and overcome any entropic barrier that might exist. The trap will be created by the interference pattern of three laser beams intersecting at a common point. By varying the relative angle of intersection of the laser beams and the wavelengths of the laser beams, the intensity maxima in the trap can be arranged to form the lattices of all crystal classes. For visible light, the intensity maxima are comparable in size to the virus. Based on the previous studies described above, the virus should be attracted to the intensity maxima. The lattice of intensity maxima can be adjusted to make the viruses touch as they would in the crystal. After the time necessary to nucleate (which may be on the order of a hundredth of a second), the trap will be removed and the newly ordered crystal nucleus is expected to maintain itself, if the enthalpic contributions are favorable.

Since viruses are rather difficult to manage and require special care, 0.2 micron dielectric spheres can be substituted for them in the initial validation of this technique. A trap would be set up to yield a known crystal nucleus, and whether it formed or not could be observed through measuring Bragg reflection angles from a fourth laser. (Of course, the spheres would disperse as soon as the laser trap is removed.) This control experiment would copy all components (solvent, container, temperature, etc) as that of the viral sample. The system could be fine-tuned and the parameters (such as duration of trapping, angle and wavelength of lasers, etc.) optimized before any virus is used. This control experiment would demonstrate that the 3D interference pattern is present and that it can impose 3D order.

Another experiment is needed to demonstrate that the virus is not physically damaged by the laser beam. This can be accomplished by setting up the trap with the predetermined parameters and simply exposing the virus to it. The virus is then introduced back into the tissue of the host. If the exposed virus multiplies at the same rate as the unexposed virus, this proves that the virus is not damaged by the 3D trapping technique. Increasing the exposure times of the trap to the virus can determine if there is an upper limit to duration of trapping without damage.

The final experiment depends on the existence of viruses in the 1000 - 2000 Angstrom diameter region that have been successfully crystallized. The crystallization parameters will be varied slightly from "success" to just until the crystals fail to form. This solution at the new conditions is then artificially nucleated; if the slight change in crystallization conditions disallowed natural nucleation, the laser-induced nucleated solution should form crystals. This would validate the laser-induced nucleation technique for viruses.

Unfortunately, if the solution with the slightly altered conditions does not yield crystals, then this is not conclusive proof that the technique is invalid. It may be that the slightly varied set of parameters is in a region where crystallization does not occur, even with artificial nucleation. It may also be that the crystal class imposed by the lasers is incorrect.

It is difficult to judge *a priori* the probability of success, but if artificial nucleation is highly successful, it could revolutionize the strategy to fight viral diseases. At a time when AIDS is spreading at an alarming rate, it might be interesting to note that the dimensions of the human

immunodeficiency virus is approximately 1200 Angstrom,[27] within the limits of this technique. Knowing the coordinates of its atoms would be quite useful in the development of an anti-AIDS drug.

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[27]Gallo, R. The AIDS Virus. *Sci. Amer.* **256**, 47-56 (January 1987).