

Letter of Intent (Category A): Imaging of Single Particles and Biomolecules at the Linac Coherent Light Source (LCLS)

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INTRODUCTION

Much of what we know about the detailed structure of biomolecules, including proteins, DNA, and RNA, has come through the use of X-ray diffraction. Conventional synchrotron radiation catalyzed revolutionary advances in this field during the past two decades, enabling the study of larger and more complex systems at increasingly high levels of resolution and on smaller (often micron-sized) crystals. The key to this great success has been the use of Bragg diffraction from the millions of oriented copies of molecules that are well aligned in a single crystal. However, there are classes of proteins (as well as many other types of materials) that are difficult or impossible to crystallize, including membrane proteins and many glycoproteins, for which structure determination at atomic resolution or even near-atomic resolution would be invaluable.

Theoretical studies and simulations predict that with a very short, very intense coherent X-ray pulse, a single diffraction pattern may be recorded from a large macromolecule, a virus, or a cell without the need for crystalline periodicity (Neutze *et al.*, 2000; Jurek *et al.*, 2004a,b; Hau-Riege *et al.*, 2004a). A three-dimensional data set could be assembled from such patterns when copies of a reproducible sample are exposed to the beam one by one (Huldt *et al.* 2003). The over-sampled diffraction pattern should permit phase retrieval and hence structure determination (Miao *et al.*, 2001; 2002; 2003; Robinson *et al.*, 2001; Marchesini *et al.*, 2003a,b, see also Miao *et al.*, 2004 for a recent review). However, the challenges in carrying out such an experiment are formidable, and will engage an interdisciplinary approach drawing upon structural biology, atomic and plasma physics, mathematics, statistics, and XFEL physics. The potential for breakthrough science is great with impact not only in the biological areas but wherever structural information at or near atomic resolution on the nanoscale is valuable.

In this Letter of Intent (LOI), we propose a seven-year program of research, diagnostic, and instrument development that will be carried out between now and the beginning of LCLS operation in 2008 and continue into the initial three years of the operations phase of LCLS. The specific aim is to develop, build, and commission a bioimaging experimental station on LCLS. This will be followed by operations to obtain the first significant results in this emerging new approach in bioimaging.

SCOPE OF WORK

It should be noted that while the conceptual design of the experiment has been studied extensively, the performance and X-ray pulse requirements of these imaging experiments will not be accurately known until validation experiments can be carried out on sources such as the DESY VUV-FEL and the LCLS itself. Therefore the initial work at the LCLS will be model-validation to determine the dynamics of the interaction of molecules and clusters with XFEL pulses, destroying the sample in the process. We will also do proof-of-concept single-shot two-dimensional (2D) imaging experiments at considerably lower than atomic resolution (yet still beyond the radiation damage limit of steady-state X-ray microscopy performed at current 3rd generation synchrotron sources). This work can be carried out with the nominal baseline LCLS pulse parameters. The research program following these initial studies will be geared toward pushing X-ray imaging to higher and higher resolution, moving from the more straightforward experiments on colloidal particles and atomic clusters, toward the most challenging experiments on single molecules, viruses and protein complexes. Our present (not yet experimentally validated) models show that atomic resolution by diffraction from single macromolecules will require XFEL pulse durations much shorter than the LCLS nominal parameters, probably less than 10 fs, while the pulse requirements for inorganic high-Z clusters will be somewhat relaxed. It is our intention to work with the SSRL/LCLS accelerator and X-ray physics efforts on reducing pulse durations by X-ray optical methods and source modification, but we will also be pursuing a path of improving resolution by several innovative X-ray imaging approaches that we propose to develop. A major component of the program will be in implementing and testing containerless sample handling technology and, to some extent, in applying particle orientation methods. These methods will be initially applied to atomic-resolution imaging of inorganic clusters and nanostructures. The ultimate goal is to bring

together all the components to image the large single biomolecules within about three years after the beginning of LCLS operations in 2008.

CONCEPTUAL DESIGN OF THE EXPERIMENTS

The experiment will consist of an apparatus to perform coherent X-ray diffraction imaging, or image reconstruction by phasing oversampled diffraction patterns. The apparatus will include optics to focus the beam onto the sample to provide the necessary X-ray fluence, and, if necessary, pulse compression optics. X-ray diffraction will occur in an ultra-high-vacuum chamber that will house sample manipulation and injection hardware, diagnostics, and a pixellated detector to record the diffraction pattern. In most experiments, a diffraction pattern will be recorded in a single pulse and the sample subsequently destroyed. A large number of diffraction patterns will be recorded, from a supply of identical or equivalent samples, and stored on a computer to be processed into a three-dimensional (3D) diffraction dataset. This dataset will be processed (phased) to obtain a 3D image of the sample. The design of the apparatus will be an evolution of chambers that we have already used for current coherent X-ray diffraction imaging experiments at SSRL/SPring8 and Stony Brook/ALS. We will also incorporate into the design of the LCLS experimental chamber the experience that we will gain at the DESY VUV-FEL high-brightness beamline, in experiments being carried out by our team between 2005 and 2008.

Components of the Experiment

Optics: The beam from the LCLS must be focused to a spot diameter on the order of 0.1 to 10 microns, depending on the overall sample size and desired X-ray fluence at the sample (initial dynamics experiments can use larger beam diameters, including the unfocussed beam). This requirement could be achieved by a Kirkpatrick-Baez mirror pair, and is best carried out in the far experimental hall to allow larger aperture optics and hence reduced fluence on the optical surfaces. The pulse duration will eventually need to be shortened, which could be achieved either by source modifications or by X-ray pulse compression, using strained crystal diffraction (Chapman and Nugent, 2002) or asymmetric multilayer gratings. Our current estimates are that < 10 fs pulses with $> 10^{11}$ photons/pulse are required for realistic single-particle imaging.

Sample Handling: The main complexity and scientific challenge of the instrument will be in sample introduction and control. Since the quantities of material of the sample under study will be minute, there should be very little other matter in the beam. When imaging single molecules, the sample cannot be held on a substrate, since scattering from the atoms of the substrate will overwhelm the signal of the molecule itself. Therefore, the sample (and indeed the entire optics and experimental apparatus) must be at ultra-high vacuum (pressure $\sim 10^{-9}$ Torr, similar to cryo-EM). Particles, such as macromolecules or virus particles, will be injected from the outside into the X-ray beam in such a way that single particles intersect with the brief XFEL pulses. Ideally, one fresh, single particle is injected into every focused pulse at a 120 Hz rate in the LCLS. To achieve this, the trajectories of the particles must be controlled both in space (< 1 micron) and time (< 10 ns), so that each one of them will be well aligned with the focused X-ray pulse. Present sample injection and particle manipulation techniques need to be significantly refined in order to position individual particles with sufficient precision. Initial experiments on particle dynamics will use clouds of particles without stringent requirements, achievable with current methods. These will be improved upon, by first injecting short, concentrated bursts of particles into the beam focus area and relying on statistical positioning of individual particles. Such experiments require shot-to-shot diagnostics to determine whether a particle was indeed hit by the X-ray beam. Such diagnostics could be provided, *e.g.*, by fluorescence detectors and by a mass spectrometer that analyzes the fragments of the particle after the shot passes through. More advanced techniques of particle introduction and manipulation include the injection of a few or even single particle into the beam at the proper time with well-controlled velocity, or trapping single particles at the XFEL beam focus using optical, electrostatic, or electromagnetic methods.

We will explore electrospray ionization (ESI) and related ink-jet spraying techniques as methods for introducing large molecules or small particles, such as viruses, into the gas phase (Gaskell, 1997). Those spraying techniques have been refined in recent years for their application in mass spectrometry of large proteins, supramolecular complexes, such as intact ribosomes (Rostom, *et al.*, 2000), and even whole viruses (Tito, *et al.*, 2000). The charge imparted onto a particle by the ESI process is convenient for manipulating the particles in the gas phase by electrostatic forces (Bogan and Agnes, 2002). If necessary, a charge reduction scheme based on the charge-reduction electrospray method (Stephenson and McLuckey, 1998) can be used to reduce the charge on electrosprayed molecular ions or particles to one or few elementary charges in a controlled way. For the particle introduction into ultra-high vacuum, we will expand on the aerodynamic lens or nozzle techniques used for single-particle mass spectrometry (Gard, *et al.*, 1997, Sues and Prather, 1999) and bioaerosol mass spectrometry developed at LLNL by some of us. We will also explore techniques based on reverse micelles or helium bubbles to provide a protective coat for the sample molecules if necessary.

Prior to LCLS operations we will develop particle orientation control methods as a means to enable single-particle imaging with less stringent pulse requirements. The ASU group will shortly perform experimental measurements of the degree of molecular alignment possible in a molecular beam by a polarized laser (Spence and Doak, 2004). They plan to use a small electron gun for diffraction to measure the degree of alignment. Simulations (Spence, submitted) show that alignment of about five degrees will resolve secondary structure in proteins about 10 nm long at 4 K in liquid helium drops.

For initial experiments, large particles such as nanocrystals of membrane-bound proteins or other materials, and cells, will be supported in vitreous ice and manipulated directly into the beam by visual microscopy. The handling of these types of samples will be upgraded to be completely containerless by using a simple electrostatic system or in-vacuum laser tweezers. This method would be ideal for diffraction imaging of membrane nanocrystals, but must be automated to enable collection of diffraction from thousands of individual crystals. In Göteborg the Neutze group has numerous on-going membrane protein crystallisation projects, and will provide nanocrystals of membrane proteins of known structure (to validate the method) and unknown structure (to achieve new science). This group will also develop the abovementioned containerless handling system.

Detectors: The diffraction pattern will be recorded on a pixellated detector subtending a solid angle dependent on the desired resolution, and a hole in the middle to avoid the direct beam. The required number of pixels of the detector depends on the ratio of the sample (or unit cell) diameter to the resolution distance, generally not less than 1000 x1000. The detector size should be approximately 50 mm x 50 mm, in order to reduce the path from sample to detector. Other desired parameters are a read-out speed per frame of 120 Hz (the LCLS repetition rate) or at least 60 Hz; a dynamic range of $\sim 10^8$ for the entire pattern (for large single particles such as viruses); a dynamic range locally of ~ 1000 (a dynamic range of 10^8 could be achievable with two detectors, each with 10^6 range where the response of the second detector measuring the strong forward scattering component is reduced with an absorber on its surface). Diffraction data may be supplemented by a lower-resolution image of the sample obtained with a zone plate as it could prove valuable in enhancing the robustness of the oversampling phasing algorithms.

Many of these requirements are in line with general directions currently driving the development of X-ray area detectors for synchrotrons and for other experiments planned for the LCLS. The detectors needed for our studies are relatively small, and that alleviates some of the difficulties in developing faster detectors for our studies. The team represented by this LOI will not be developing the diffraction detector, but we will collaborate with laboratories and companies who will do so.

Image Reconstruction: As in crystallography, the computer is a major instrument component and algorithms are required to generate images from the measurements. Single-shot 2D diffraction patterns of 1 to 10 micron diameter objects such as cells, at resolutions above the radiation damage limit, will be reconstructed straightforwardly using methods proven by many of us and for which references have been provided in the Introduction. Three-dimensional imaging will take place in two distinct stages. In the first stage, the relative orientation of a large number of noisy 2D diffraction patterns must be determined in 3D reciprocal space and then merged into a single 3D diffraction data set (Huldt *et al.*, 2003). In the second stage, phases are derived from the 3D diffraction pattern using an iterative algorithm. Two such approaches have recently been described (Miao *et al.*, 2001; Hau-Riege *et al.*, 2004b). The main computational burden is expected to be in the first stage. While methods for classification of images that have been developed for cryo-EM (Frank, 1996; van Heel *et al.*, 2000) could be applied here, the differences between the methods require further studies in this area. A new method has been proposed (Elser, 2004) which determines the relationship in the continuous Euler angle space of each diffraction pattern as it is acquired. This method could in principle be applied in real time using a distributed computer cluster, and has the ability to determine when a complete dataset has been collected (*i.e.*, when to stop collecting data). For the second stage, methods will be developed in applying oversampling techniques to phasing diffraction from 2D and 3D nanocrystals, and in applying *a priori* information that may be available in the diffraction of clusters and aligned particles.

RESEARCH PLAN

Before LCLS becomes operational in 2008, we plan to conduct experimental measurements at two other linear-accelerator-based X-ray sources: the VUV-FEL at DESY and the Sub-Picosecond Photon Source (SPPS) at SLAC. In developing the LCLS biological imaging experiment, we will thus be building upon our experience on these sources, as well as on preparatory work at synchrotron facilities and in our laboratories. Several groups on our team have been granted beamtime at the VUV-FEL through a competitive call for proposals and approval of a proposal that received a very high ranking. The VUV-FEL program of research

has been designed to confront the experimental challenges of single-particle imaging at the LCLS, and to carry out the first possible validations of models to enable us to plan and design the LCLS instrumentation. Briefly, the experiments at the Hamburg VUV-FEL will include: measurement of the Coulomb explosion of inorganic and biological particles irradiated by ultrashort VUV pulses (for earlier studies on TTF-I see Wabnitz et al. 2002; Laarman et al. 2004); demonstrations of single-shot diffraction imaging of biological samples at resolutions beyond the steady-state X-ray damage limit; and demonstrations of 3D diffraction imaging of reproducible test samples. In addition, diagnostics to measure pulse structure will be tested and applied to studies on the control of explosions using dual pulses. By the end of the third year of VUV-FEL experiments, in 2007, we will have demonstrated techniques to inject clouds of particles, and single particles, into an FEL beam and measure their coherent diffraction patterns; tested ideas to trap and orient particles; determined methods to work with fully spatially coherent beams (*e.g.* to eliminate coherent noise and parasitic scattering from beamline components and gas molecules); tested sample preparation concepts to boost diffraction signal levels; and have an understanding of the required pulse parameters for LCLS experiments.

Timeline at LCLS and Expected Results

Experiment	Description	Timeline
Dynamics of particles and clusters	Determine damage rates and test damage control strategies	2008-2011
2D single-shot imaging	Zone-plate and diffraction imaging beyond radiation damage resolution limits	2008-2009
Imaging inorganic clusters	Diffraction imaging inorganic colloids and clusters	2009-2010
3D imaging of membranes	Diffraction imaging of membrane nanocrystals	2010-2011
3D imaging of particles	Diffraction imaging of aligned and non-aligned particles, including viruses	2010-2011

A summary of experiments is given in the above table. The experimental program at LCLS will roughly follow the order of the VUV-FEL experiments due to the progression in complexity and the dependencies of the experiments. Also, the timeline is driven by the development of diagnostics, sample handling such as particle positioning, orientation, alignment, and X-ray optics or source upgrades for reduction of the pulse duration. The broad timeline and description of experiments is as follows:

2008: Dynamics of particles and clusters. Atomic-resolution imaging of single particles depends crucially on the rate of damage induced by photoionization by the XFEL pulse. Until we perform experiments at LCLS, there will be no validation of models in the high-energy density regime and required imaging conditions. The results from these measurements will guide the imaging experiments that follow. Initial dynamics experiments will measure XFEL diffraction from a cloud of particles, as a function of pulse parameters (most importantly, pulse fluence). The diffraction pattern will give the time-integrated structure factor of a single particle, modulated by speckle due to the random positions of the clusters or particles. Initially we are primarily interested in the average structure factor, which will give us information on the size and density profile of the particles. These initial measurements will be performed with an off-axis detector (*i.e.* to avoid the direct beam). As we reduce particle density we will record a full diffraction pattern that can be phased by oversampling (to get an image of one or several particles). We will study mechanisms to slow down the explosion, such as the use of a helium-drop tamper (Hau-Riege *et al.*, 2004b), or a modified pulse structure. Theoretical studies on the control of the dynamics of the explosion of hydrogen clusters by ultra-intense laser pulses (Fajardo *et al.*, 2004; Peano *et al.*, 2004) suggest that the relative intensities and delays between two pulses could be used to create “designed” expansion geometries and times. In addition to collecting the diffraction data we will perform TOF mass spectrometry on the fragments of the particles or clusters. The outcome of these experiments will give an improved knowledge of the explosion of X-ray irradiated samples and help in developing approaches to control the rate of these events. While searching for the optimal conditions for biological X-ray diffraction imaging, we will be inspecting unprecedented interaction regimes, with tremendous potentials in atomic physics and high energy-density science.

2008-2009: 2D single-shot imaging. Initial imaging experiments will be performed at low resolution and low photon energy and fluence, using a zone plate lens. These experiments will be primarily to commission the LCLS instrument and beamline, to characterize beam intensity profile and coherence, and to eliminate sources of unwanted scattering. The sample will be on a membrane in a cryo-holder and will be positioned in the beam with a visible microscope system. Note that the cryogenic sample holder is not for radiation protection, just a convenient way to mount the sample. In 2009 experiments will move to higher fluence, where the sample will be destroyed on irradiation, and to higher resolution, using diffraction imaging. The initial goal is to achieve better than 10 nm resolution on cellular materials. This is at or slightly better resolution than what could be achieved with steady-state X-ray microscopy of cryogenic biological samples

(Miao *et al.*, 2004). We then plan to continue resolution improvements on smaller samples as we move to shorter pulses in the next phase of experiments.

2009-2010: Imaging inorganic clusters. The geometric structure of clusters is of fundamental importance for the understanding of many other properties e.g. their electronic structure, reactivity, magnetic, and optical properties. Most information on the geometric structure of clusters comes from indirect methods e.g. mass spectroscopy (Haberland, 1993, Martin *et al.*, 1990), flow tube measurements (Jarrold, 1991) and from theoretical work (Röthlisberger *et al.*, 1994). The LCLS biological imaging experiment will allow atomic-resolution X-ray diffraction analysis, which could not be applied as yet since the particle density of cluster in a beam is very low. The pulse requirements for imaging high-Z materials may be much less stringent (our simulations estimate ~50 fs pulses in a 300 nm diameter spot) than for biological materials, due to the increased scattering cross section and certain translational symmetry and crystallinity that results in very intense Bragg-like diffraction peaks. We will therefore be able to develop the sample handling and image reconstruction techniques that are required for biological imaging at these relaxed pulse parameters. We will perform initial demonstrations of the classification and reconstruction from coherent diffraction at unknown orientation, which will be achieved first at lower resolution on electrosprayed and evaporated drops of colloidal particles.

Particularly interesting are studies on clusters which undergo structural phase transitions like gold clusters (Cleveland *et al.*, 1997) or alloyed clusters which could provide an opportunity to observe the competition between atomic segregation and stoichiometric mixtures as the function of size and temperature (Maier-Borst *et al.*, 1999). Depending on the geometrical structure, the size and of the temperature of the clusters different vibrational modes can be studied. This will be particularly interesting for systems that show strong variations of the melting temperature with size like S_n clusters (Shvartsburg and Jarrold, 2000). Furthermore, time-resolved structural studies will allow following the pathway of photo-induced reactions in real space. The advent of direct time-resolved structural probes will certainly open new scientific opportunities in this field which had to rely in the past mainly on indirect measurements.

2010-2011: Imaging biological nanocrystals. The pulse-length requirements for atomic-resolution imaging of biological materials can be much relaxed by using methods of imaging molecules in parallel. One such parallel method is to diffract from arrays and crystals of particles, and in particular XFEL diffraction of membrane nanocrystals would significantly impact structural biology. 25% of the human proteome encodes membrane proteins but only 1% of the available structural information relates to membrane proteins. The LCLS experiments will be performed using the automated nanocrystal manipulation techniques described above. The 8 keV photon-energy LCLS beam will be focused to a 1-micron diameter spot or smaller, and diffraction recorded from a single membrane protein nano-crystal (destroying the crystal in the process). The image reconstruction techniques will be analogous to the single-particle case, and will require thousands of diffraction patterns from randomly oriented crystals to obtain a complete dataset. The orientation of the crystal will not be preset, but will be determined in part by indexing the diffraction pattern. In the case of 2D crystals, individual Bragg truncation rods can be phased using iterative methods as demonstrated by Spence *et al.* (2003). In the X-ray diffraction case, where a 2D image is not available, phase relationships of the truncation rods may possibly be determined by a highly redundant fit of a 2D sinc-function expansion of the phased rods to weak off-Bragg diffraction (dependent on crystal shape).

The scientific impact of a "short cut" to better resolution X-ray structures of membrane proteins is tremendous for science and medicine. In particular, the LCLS will significantly out-perform conventional micro-focus synchrotron beamlines, which themselves have been essential for the recent progress in membrane protein structural biology.

2010-2011: 3D imaging of biological particles.

Another method of parallel imaging to relax XFEL pulse requirements is diffractive imaging from a beam of aligned, hydrated molecules. Alignment could be achieved with existing continuous lasers, and may provide sufficient resolution in reconstructions to show secondary structure in proteins. Initial calculations (Spence *et al.*, submitted) indicate that at 4K an alignment error of about 5 degrees could be achieved this way. The diffraction pattern from the aligned cloud of particles would be the continuous molecular transform of a single molecule (Szöke, 1986) modulated by speckle that will be of higher spatial frequency than the detector pixel pitch and not be detected. That is, single-particle over-sampling techniques can be applied without modification to reconstruct images. While such methods could be applied with long integration times with sources such as a synchrotron or an electron gun (Spence and Doak, 2004), the LCLS offers the opportunity to enhanced resolution and also to perform time-resolved studies on molecules that are not constrained in a crystal. The initial experiments will be performed with a 1-micron diameter beam and particles will be injected into the focal volume. These experiments will progress to smaller numbers of particles per pulse as the pulse duration is reduced, and eventually to single-molecule imaging without the

requirement of optical alignment. In 2011 we aim to perform diffraction imaging experiments on single non-aligned virus particles, such as the cow-pea mosaic virus (Wang *et al.*, 2002).

By the conclusion of these experiments, and with development of pulse compression techniques or source upgrades, we will have an instrument that will be capable of determining atomic- or near-atomic resolution of 3D structures from nanocrystals and large single biomolecules. When successful, this will truly have a large impact on structural biology, allowing structures to be acquired of materials that have not been amenable to crystallization or other methods. It will also open new approaches to study of dynamics through synchronization of structural studies with laser-initiated reactions.

OVERVIEW OF THE PROJECT TEAM, MANAGEMENT, AND FUNDING

The applications of X-ray free-electron lasers (XFELs) have been examined in a series of workshops and reports both in the USA and abroad. References and details of the many workshops and reports related to the scientific cases for the two major X-ray FEL projects in the world can be found on the www sites of these two facilities at www.ssrsl-slac.stanford.edu/lcls/ and http://xfel.desy.de/content/e169/index_eng.html A number of new scientific opportunities have been identified and elaborated, several of which are in the area of structural biology. The concept for atomic-resolution imaging using X-ray pulses from an X-ray FEL like LCLS arose from the convergence of the ideas of coherent X-ray diffraction imaging and overcoming the limitations of the radiation damage by the method of “flash imaging” with very short and very intense X-ray pulses. These ideas came together in the forums defining the scientific cases for the LCLS and TESLA XFELs, held in the mid-to-late 1990’s, where the experimental concepts were initially defined. In parallel, concepts such as methods for delivering particles into the beam and classifying diffraction patterns of particles of unknown orientation have also evolved. A comprehensive view of biological imaging experiments at the LCLS and the R&D needs were developed at a recent workshop (“Instrument Development Workshop for Biological Imaging at the LCLS”, held at SLAC/SSRL in March 2004). The research proposed in this LOI is based on these efforts, and focuses a specific set of goals to achieve the ultimate success of single molecule imaging with the LCLS.

Roles and Responsibilities of Team Members. The LCLS biological imaging program has been guided by a steering committee consisting of Professor Janos Hajdu (Chair, University of Uppsala), Dr. Henry Chapman (LLNL), Professor Carol Robinson (Cambridge University) and Professor Keith Hodgson (SSRL and Stanford). The Table below lists the expertise and individual role of all participants in the project:

NAME	INSTITUTE	ROLE / EXPERTISE
Janos Hajdu	UPPSALA	PI, theory, diffraction imaging, sample preparation
Keith Hodgson	SSRL/SLAC	Co-PI, phasing, detectors, FEL-science, SSRL, SPPS
Henry Chapman	LLNL	Co-PI, optics, diffraction imaging
John Miao	SSRL/SLAC	Phasing, imaging, nanocrystals
Jochen Schneider	DESY	FEL-science, VUV-FEL, detectors, clusters, DESY facilities
Josef Feldhaus	DESY-VUV-FEL	FEL-science, lasers, VUV-FEL experiments
Elke Ploenjes	DESY-VUV-FEL	FEL-science, lasers, VUV-FEL experiments
Edgar Weckert	DESY	Coulomb explosions, detectors, FEL-science, PETRA
Ivan Vartianians	DESY	diffraction physics, reconstruction, coherence
Christian Schroer	DESY	micro focussing optics, X-ray imaging, microfluorescence
Ian McNulty	APS	Optics, imaging
Janos Kirz	LBNL	Imaging
Musahid Ahmed	LBNL	Sample preparation and injection, mass spectrometry
Hamed Merdj	CEA	Soft X-ray lasers tests
Philippe Zeitoun	LOI	Soft X-ray lasers tests
David van der Spoel	UPPSALA	Theory, damage, clusters, software
Nicusor Timneanu	UPPSALA	Theory, damage, clusters, software
Martin Svenda	UPPSALA	Sample preparation
Gosta Huldt	UPPSALA	Imaging and image reconstruction
Carl Caleman	UPPSALA	Damage models, timing, imaging
Magnus Bergh	UPPSALA	Damage models, laser experiments
Sara Lejon	UPPSALA	Sample selection and injection, diffraction imaging
Alexandra Patriksson	UPPSALA	Theory, software, laser experiments

Veit Elser	CORNELL	Image reconstruction, phasing, software development
James Fienup	ROCHESTER	Phasing
Lukas Novotny	ROCHESTER	Sample trapping and orientation
Pascal Anger	ROCHESTER	Sample trapping and orientation
Jan Isberg	UPPSALA	Damage, electron cascades in covalent carbon structures
David Sayre	STONY BROOK	Phasing
Chris Jacobsen	STONY BROOK	Imaging, instrumentation
David Shapiro	STONY BROOK	Imaging, instrumentation
Huije Miao	STONY BROOK	Imaging, instrumentation
Enju Lima	STONY BROOK	Imaging, instrumentation
Helmut Strey	STONY BROOK	Sample delivery
Richard Neutze	CHALMERS	Nanocrystal diffraction and preparation
Arjan Snijder	CHALMERS	Nanocrystal diffraction and preparation
Susanna Tornroth	CHALMERS	Nanocrystal diffraction and preparation
Ian Robinson	URBANA	Imaging, nanocrystal diffraction
Roger Falcone	BERKELEY	Carbon nanotubes, damage studies, imaging
Thomas Möller	BERLIN	Imaging of clusters, damage models
Christoph Bosted	BERLIN	Imaging of clusters, damage models
John Spence	ASU	Sample injection, orientation, imaging
Martha Fajardo	LISBON	Clusters, damage studies, Soft X-ray lasers,
Nelson Lopes	LISBON	Clusters, damage studies, Soft X-ray lasers,
Joao M Dias	LISBON	Clusters, damage studies, Soft X-ray lasers,
Goncalo Figueira	LISBON	Clusters, damage studies, Soft X-ray lasers,
Luis Silva	LISBON	Software development, explosion control
Ricardo Fonseca	LISBON	Theory, explosion control
Fabio Peano	LISBON	Theory, explosion control
Beata Ziaja	KRAKOW	Theory, cascades, damage control
Gyula Faigel	BUDAPEST	Holographic techniques, phasing, damage studies
Keith Nugent	MELBOURNE	Optics, imaging
Carol Robinson	CAMBRIDGE	Sample selection, injection, diagnostics
Eugene Ingerman	CBS	Diffraction imaging
Stefan Hau-Riege	LLNL	Coulomb explosions, damage control
Hope Ishii	LLNL	Damage, imaging, diagnostics
Stefano Marchesini	LLNL	Diffraction imaging
Abraham Szöke	LLNL	Holographic techniques, phasing, imaging
Peter Young	LLNL	Laser education, Coulomb explosions
Richard Lee	LLNL	Coulomb explosions, clusters
Richard London	LLNL	Coulomb explosions, clusters
Rodney Balhorn	LLNL	Sample preparation
Henry Benner	LLNL	Sample selection, injection, orientation
Matthias Frank	LLNL	Sample selection, injection, orientation
Aleksandr Noy	LLNL	Carbon nanotubes, sample preparation
Anton Barty	LLNL	Diffraction imaging and instrumentation
Brent Segelke	LLNL	Sample preparation, diffraction imaging
Daniel Barsky	LLNL	Theory, sample injection

Management, Funding, and Financial Considerations. This experimental program on single particle imaging of biomaterials engages an existing team of about 65 collaborating scientists from 9 countries as listed in the beginning of this LOI and in the table above. The effort of this instrument development team (IDT) will be coordinated and managed through a steering committee with representation from Uppsala University (Hajdu, PI), LLNL (Chapman, co-PI), SSRL (Hodgson, co-PI) and Oxford (Robinson) and manpower will be expanded as the effort ramps up. It is anticipated that our IDT will interface with SSRL/LCLS to obtain appropriate support in developing detailed designs and engineering to ensure that the experimental system will be fully compatible with, and integrated into, the LCLS experimental program. As a result of the workshop described above on the LCLS bioimaging experiment, we have developed a preliminary conceptual instrument concept and cost. The major elements of this are listed below and for

each is given an overall capital/operations cost (excluding manpower) and a manpower estimate (number of FTEs). The estimates are for *total effort over the 7 year period*. Manpower estimates include engineering/design, scientific and technical/assembly and experimental staffing: The 6 elements are: 1. X-ray optics and experimental front end – includes final focusing, zone-plate imaging, and pulse compression - R&D, \$1,500K, 14 FTE; 2. Sample injection and handling – includes R&D and methods for particle and nanocrystal preparation and injection, orientation (magnetic and optical) - \$2,550K, 47 FTE; 3. Chamber and Diagnostics – includes R&D at the VUV-FEL and SPPS, equipment for beam characterization and monitoring, pulse diagnostics, integrated visible microscope, mass spectrometer, electron spectrometer, and sample stages - \$3,600K, 35 FTE; 4. Detectors – includes two 2D pixel array detectors and associated readout electronics - \$2,000K, 2 FTE; 5. Simulations and theory – includes effort for reconstruction development, software and integration, and compute and storage subsystems - \$350K, 66 FTE; 6. Other – includes laboratory equipment for sample preparation (visible and electron microscopes, centrifuge, cold room), fs-pulsed laser (microJ/pulse) - \$2,600K, 3 FTE. Elements 1. through 6. sum to \$12.60M and 167 FTEs over 7 years.

We anticipate building upon funding that is already being committed (or sought in pending applications) for aspects of this imaging program by the participating institutions. SSRL, through core research funds and grants (new and pending) from NIH and DOE-BER will contribute \$1.27M and 42 FTEs. The University of Uppsala will contribute 56 FTEs through existing and submitted Swedish and EU grant applications. We anticipate collaborations with LCLS and with DESY to lead to the detector, but include 2 FTE in this project cost for integration. This leaves \$9.33M in equipment/other costs and 69 FTEs. Assuming an average cost of \$200K/FTE, this gives a remaining total needed over the seven years of \$23.13M (\$9.33M + \$13.8M). LLNL is seeking strategic laboratory initiative (internal) funds for \$5M of this total. The remainder will be sought through the development and submission of DOE proposals of \$18.13M (~\$12.03M to DOE-BES and ~\$6.1M to DOE-BER). Alternative sources of funding could include the NSF or private 3rd party investments.

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