Three important frontiers of ultrafast time-resolved macromolecular crystallography are presented: extension of this technique to other biological systems; further developments in the elucidation of mechanism through the analysis of time-dependent movies to extract the underlying, time-independent, intermediate structures; and enhanced time resolution. The last is intimately linked with the nature of the pump–probe experiment itself, with the sources of random and, particularly, systematic experimental error, and with the factors that contribute to overall time resolution. All experiments to date have utilized the unchirped X-ray pulses that are emitted by synchrotron sources. Chirped pulses offer certain advantages for ultrafast X-ray experiments such as those based on Laue diffraction. An energy-chirped pulse maps photon energy into time; a Laue diffraction experiment maps photon energy into detector space. Hence, a Laue experiment with an energy-chirped pulse maps time into space. The proposed sub-picosecond photon source could provide an excellent source of intense, chirped hard X-rays for such experiments.

Introduction

In time-resolved macromolecular crystallography, a structural reaction is initiated at a defined time in the molecules in a single crystal at near-room temperature, where full structural transitions are possible. The reaction progress is monitored through the subsequent time dependence of the X-ray structure amplitudes, \( |F(hkl, t)| \). The ultimate goal of such an experiment is to identify the structural mechanism: that is, to determine the structures of reactants, of all intermediates and of products; and the pathways and rates by which these structures interconvert.\(^1\) This is challenging. The differences between these structures are expected in general to be small; accurate measurements and high crystallographic resolution are necessary to reveal them. Certain intermediate structures may have very short lifetimes and interconvert rapidly; excellent experimental time resolution is required. Yet others may be long-lived; data must be collected over many decades in time. Unless the reaction mechanism is simple and the magnitude of the rate coefficients for the structural interconversions particularly favorable, the crystal contains, at all times, a time-dependent mixture of structural states; this mixture must be resolved. That is, the time-independent structures of each intermediate must be extracted at the data analysis stage, “analytical trapping”. This overall, time-resolved approach may be contrasted with other crystallographic approaches that seek to isolate and study homogeneous, intermediate structures by manipulation of the chemistry of the system, “chemical trapping”, or by lowering the temperature, “physical trapping”.\(^2,3\)
It is clear that successful ultrafast, pump–probe, time-resolved experiments can be conducted, in which a light-driven reaction is initiated or pumped by a brief laser pulse and the X-ray diffraction pattern is acquired or probed after a suitable time delay by a brief X-ray pulse. This paper explores briefly three of the frontiers of such experiments, then describes how a novel type of chirped X-ray pulse might reduce systematic experimental errors and aid in attacking the third of these frontiers, time resolution.

**Frontiers**

(a) Biological systems

To date, ultrafast time-resolved experiments have been published on only two systems, myoglobin and photoactive yellow protein. A first and obvious frontier is therefore to apply the technique to light-sensitive systems of wider biological interest. Light directly influences many organisms, for example through light-harvesting complexes harnessed to efficient photosynthesis, and through light-driven signal transduction. In the latter, photons of a particular wavelength range and intensity are absorbed by a detector molecule or domain, a signal is generated and transmitted in both an intramolecular and intermolecular fashion to at least one output molecule or domain, and a measurable output is produced. Examples of such outputs include altered swimming behavior of bacteria, or phototropism in plants. The molecular and structural bases for the light-driven generation and transmission of signals are not well understood. The recent availability of, for example, the complete genome sequence of the experimental plant Arabidopsis enables many such light-sensitive systems in the plant to be cataloged. The Arabidopsis genome encodes at least 58 proteins that respond to light, including 9 that are homologous to known signal transduction proteins. Each of these 9 proteins represents an upstream component; each must interact with other, downstream components, many of which are kinases, to form an entire signal transduction pathway. Light-dependent structural studies of the members of a pathway will reveal how the initial structural signal is generated, and how it is transmitted.

As one specific example, the blue-light photoreceptor known as phototropin controls phototropism, chloroplast relocation and other processes in plants. Absorption of a blue photon by the FMN chromophore in a small domain of phototropin known as the LOV domain leads to the rapid but transient and fully reversible formation of a covalent bond between a cysteine side chain in the FMN pocket and C(4a) of the FMN. Phototropin also contains an integral serine–threonine kinase, which confers autophosphorylation activity on phototropin that is modulated by the structure of the LOV domain. Thus, phototropin contains a light-driven molecular switch, the LOV domain, which confers light sensitivity on an enzymatic domain, the kinase. The structural basis for this is completely unknown.

(b) Determination of the mechanism: from movies to time-independent intermediates

How shall “analytical trapping” best be conducted, to establish whether a chemical kinetic mechanism with well-defined intermediate states exists? If it does exist, how shall the time-independent structure associated with each state be cleanly extracted and refined? Answering these questions constitutes a second frontier.

Variations in the magnitude of the features in time-dependent (difference) Fourier maps arise from variation in the concentrations of the intermediates. These variations may be modelled and realistically presented as “movies” (see for example ref. 4 and 5) in which the electron density varies smoothly with time. However, interest lies not so much in the smooth variation of concentrations, but in the nature of the individual, distinct, intermediate structures. Progress along the reaction coordinate is not smooth, but may be visualized as a “hopping” of molecules from basin to basin in a complex energy landscape, in which each basin is associated with an intermediate state of the system. Molecules do not spend significant time between basins. In particular, their residence time in the transition states that are represented by the mountain passes in this energy landscape lies in the femtosecond range. The best representation of reaction progress is therefore not a smoothly varying movie; it is a set of time-independent intermediate states or structures, and the accompanying rate coefficients for their interconversion. However, the number of such
intermediates, how they interconvert and the time courses with which their concentrations vary are not known \textit{a priori}. These quantities must be extracted from the time-resolved crystallographic data, and constitute determination of the mechanism.

If the mechanism is simple, then direct inspection of the “movie” may reveal it,\textsuperscript{4} but a more general approach is needed. A promising strategy is to examine simultaneously the variation of the (difference) electron density at all grid points for all time points, using the mathematical tool of singular value decomposition, SVD.\textsuperscript{12} SVD has been widely and successfully used for many years, for example to separate the time and wavelength variables in time-resolved absorption spectroscopy (for an example, see ref. 13) and, more recently, to analyze on a genome-wide basis the patterns of temporal expression of genes.\textsuperscript{14} SVD can be readily adapted to the analysis of time-dependent (difference) electron density maps. SVD analysis yields a set of singular values, the species-associated (difference) electron density distributions or maps, each identified with a particular singular value, and the time course with which each distribution varies.

SVD is playing three main roles.\textsuperscript{12} First, it separates the time and structure variables. Second, it acts as a noise filter which is “best” in a least-squares sense; and this property can be employed in an iterative mode to improve phase information (M. Schmidt \textit{et al.}, unpublished results). Third, the time courses can be fitted by suitable functions such as a sum of exponentials, the exponents of which reveal macroscopic rates; and the species-associated distributions may be separated into a sum of underlying distributions, each of which arises from a desired, time-independent intermediate. The relationship between the species-associated distributions and the intermediates, and between the macroscopic rates and the microscopic rate coefficients, constitutes the mechanism.

 Preliminary results on both mock and real data (M. Schmidt \textit{et al.}, unpublished results) suggest that this is a promising approach to the elucidation of mechanism.

\textbf{(c) Enhanced time resolution}

A substantial challenge is offered by experiments that seek to examine very fast processes in the fs to 100 ps time range. Typically, the processes are light-sensitive and their initiation is readily obtained by illumination of the sample with a short, high power, laser pulse. Experiments in the fs time range are particularly attractive since ultrafast structural dynamics in condensed matter occur on the time scale of the period of an interatomic vibration, below or around 100 fs. For example, certain important biological processes such as photosynthesis have their origin in events that occur on the fs time scale.\textsuperscript{15} As discussed in more detail below, these processes are experimentally inaccessible with much longer, synchrotron X-ray pulses of around 100 ps duration. Enhancing the time resolution into the fs range to elucidate the nature of these processes thus constitutes a third frontier.

In considering how to attack this frontier, the limitations of pump–probe experiments should also be taken into account.\textsuperscript{1,4} The time-resolved X-ray structure amplitudes $|F(hkl,t)|$ have to be acquired in a four-dimensional space spanned by the three variables in reciprocal space and by time. Changes in these structure amplitudes with time are small and error-prone. Reference values of these amplitudes $|F(hkl,0)|$ must be obtained prior to the initiation of any structural reaction if accurate measurements are to be made of the differences in structure amplitudes in time, $|F(hkl,t)| - |F(hkl,0)|$. Radiation damage limits the amount of data that can be acquired on any one crystal, which introduces systematic errors through crystal-to-crystal variation. If, as has been the case in experiments to date, the $(hkl)$ variables are scanned rapidly and time is scanned slowly, then the evolution of the structural process must be pieced together from separate measurements made at different values of $t$, often on different crystals. Since each time point requires a separate reaction initiation, comparison of these measurements to extract the time-dependent signal is inaccurate and error prone. Further errors will be introduced if the probe X-ray pulse is destructive, since each time point now requires a separate crystal, not merely a separate measurement. Finally, it is a rule of thumb that the faster the structural reaction, the more limited are the underlying structural changes. Thus if the time resolution is improved, the precision of the structural measurements must also be improved.

An experimental strategy that both offers enhanced time resolution and minimizes systematic sources of error has appeal. Chirped X-ray pulses offer one such strategy.
Chirped X-ray pulses

In an unchirped pulse of electromagnetic radiation, the spectrum and direction of propagation are constant across the pulse and do not vary with time (Fig. 1a). In contrast, in a chirped pulse, a property of the radiation such as its energy or direction of propagation varies across the pulse and hence depends on time. For example, in a simple form of energy-chirped pulse the spectrum is monochromatic at all time instants but varies between a minimum energy $E_{\text{min}}$ at the earliest, leading edge of the pulse and a maximum energy $E_{\text{max}}$ at the latest, trailing edge (Fig. 1b). In a spatially-chirped pulse, the spectrum is again monochromatic but is constant in time. However, the direction of propagation of each ray varies with time and thus the rays incident on a sample sweep out a fan of radiation: the angle of incidence of the earliest ray differs from that of the latest ray (Fig. 1c). A pulse may combine features of an energy chirp and a spatial chirp, in which both the spectrum and the direction of propagation vary with time (Fig. 1d). The key feature of a chirped pulse is that it provides intrinsic time-tagging of photons: there is a one-to-one correspondence between a given energy or direction of propagation of photons and their temporal location in the pulse.

Chirped pulses are widely used in the microwave and visible regions of the spectrum. For example, the amplification of chirped pulses and their compression in time forms the basis for all high power, short pulse lasers.\(^{16}\) However, chirped pulses have not been utilized in the hard X-ray region of the spectrum. Such pulses are not readily available, since synchrotron beamlines normally deliver almost entirely unchirped pulses.\(^{17}\)

In the following sections, I point out that chirped hard X-ray pulses are particularly well-suited to ultrafast X-ray experiments with high time resolution, and discuss their applicability to single crystal Laue diffraction experiments in some detail. The proposed sub-picosecond photon source,

![Fig. 1 Unchirped, energy- and spatially-chirped pulses. (a) Schematic of an unchirped pulse. All instants in the pulse contain the same range of X-ray energies $E_{\text{min}}$ to $E_{\text{max}}$. (b) Schematic of a simple energy-chirped pulse. The X-rays are monochromatic at all instants in the pulse, but this energy varies from $E_{\text{min}}$ at the earliest, leading edge of the pulse to $E_{\text{max}}$ at the latest, trailing edge of the pulse. (c) Schematic of a simple spatially-chirped pulse. The X-rays are monochromatic with energy $E$ at all instants but their direction of propagation i.e. their angle of incidence on the sample varies across the pulse. (d) Schematic of a combined energy- and spatially-chirped pulse. Both the energy and the angle of incidence of the pulse on the sample vary with time.](image-url)
SPPS, a precursor to the LCLS at Stanford, could be an excellent source of chirped hard X-ray pulses.

**Ultrafast time-resolved X-ray experiments**

(a) **Time resolution**

High time resolution is usually achieved by one of two routes: using a time-slicing X-ray detector such as a streak camera that directly provides a time resolution around 1 ps\(^{18}\) or by conducting the experiment in pump–probe mode, in which the evolution of the processes after a laser, pump pulse is monitored at a suitable and variable time delay \(t\) by an X-ray, probe pulse.\(^{4,5}\) However, there are limitations on both routes. The active area of streak cameras is small and this restricts the classes of experiments to which they can be applied. They are either zero-dimensional, recording the time dependence of the flux of X-rays falling on one pixel, or one-dimensional, recording the flux on a short linear array of pixels. No ultrafast, time-slicing, two-dimensional detector exists. If it did, measurements could be made on the same sample, continuously in time.

The time resolution \(\Delta \tau\) of conventional pump–probe experiments is set by three contributions: the duration of the pump laser pulse \(\Delta t_L\) that initiates the structural process, the duration of the probe X-ray pulse \(\Delta t_Z\), and the jitter in the time delay between the two pulses \(\Delta t_J\), eqn. (1):

\[
\Delta \tau = (\Delta t_L^2 + \Delta t_Z^2 + \Delta t_J^2)^{1/2}.
\]

It therefore appears that achieving a value of \(\Delta \tau\) around 100 fs requires that each of \(\Delta t_L\), \(\Delta t_Z\) and \(\Delta t_J\) be significantly lower than that value. It is non-trivial to achieve values of \(\Delta t_J\) much below 1 ps. Although ultrafast laser pulses with values of \(\Delta t_L\) in the tens of fs time range are readily obtained, this is not the case for X-ray pulses. The X-ray pulse length \(\Delta t_Z\) emitted by near-circular synchrotron sources is determined by the electron bunch pulse length, which in turn is limited to 50 to 100 ps by essential, long-term stability requirements on the electron beam. Considerable effort has therefore been put into the development of novel fs hard X-ray sources. Examples include laser-driven plasmas\(^{19}\) and diode sources,\(^{20}\) and sources based on high order harmonic generation\(^{21}\) or interaction between a laser pulse and a relativistic electron beam.\(^{22}\) However, at present the X-ray flux per pulse that these sources can deliver to the sample is too low to be experimentally useful for much more than proof-of-principle experiments.

A very promising, proposed source is the hard X-ray free electron laser, XFEL. In this device, extremely bright, fully spatially and temporally coherent, 100 fs pulses of X-rays are to be generated by the self-amplified spontaneous emission or SASE process\(^{23}\) as a brief electron pulse derived from a linear accelerator traverses a very long undulator of roughly 100 m in length. However, the X-ray pulses from an XFEL are associated with electric fields believed to be sufficiently high to cause full ionization of all atoms and the complete destruction of the sample,\(^{24}\) and the SASE process has not yet been demonstrated at hard X-ray wavelengths. No decision has yet been made on the proposal to construct the first such device at Stanford Synchrotron Radiation Laboratory, to be known as the Linear Coherent Light Source, LCLS.

Chirped X-ray pulses provide a third route to obtaining high time resolution. What is required is not necessarily a 100 fs X-ray pulse but rather, a much longer, non-destructive X-ray pulse at the sample whose characteristics still permit excellent time resolution. Although X-ray experiments and detectors cannot readily distinguish photons in time, they can easily distinguish photons in energy or in direction of propagation. The time tagging of photons inherent in a chirped pulse maps time into energy in an energy-chirped pulse, or into direction of propagation in a spatially-chirped pulse. The time variable is established by the nature of the chirp. In essence, chirped pulses map a variable that cannot be readily measured, time, into a variable that can be more easily measured, energy or direction of propagation.

(b) **Time-resolved single crystal Laue diffraction**

A straightforward example of how a chirped X-ray pulse would be utilized is provided by an ultrafast time-resolved X-ray diffraction experiment.
When a polychromatic, quasi-parallel pulse of X-rays falls on a stationary single crystal, a Laue diffraction pattern is generated that consists of an array of diffracted X-ray beams. Each beam is characterized by the set of crystal planes \((hkl)\) that give rise to it, by its direction with respect to the incoming beam, by its intensity and by its X-ray energy. When the beams fall on an area detector such as a CCD or image plate, they generate an array of spatially-separated spots, the Laue spots, that comprise a single Laue image. It is straightforward to derive the unit cell dimensions and crystal orientation from the locations of the spots, and hence to index each spot \((hkl)\) and identify the energy that stimulates it. That is, Laue diffraction automatically maps the energy of each spot into its spatial location (Fig. 2). The X-ray structure amplitudes \(|F(hkl)|\) can also be obtained by measurement of the integrated intensity of each spot, and the derivation and application of energy-dependent correction factors.

As described above, time-resolved Laue experiments in macromolecular crystallography have been conducted in pump-probe mode. The experiments yield the time dependence of the structure amplitudes \(|F(hkl,t)|\) that arises in response to a structural perturbation which alters the spatial distribution of electrons in the unit cell but leaves the crystal lattice unchanged. When the X-ray phases associated with each value of \(|F(hkl,0)|\) are known, the time-dependent, space average electron density distributions \(\rho(xyz,t)\) and the time-dependent difference electron density distributions \(\Delta \rho(xyz,t) = \rho(xyz,t) - \rho(xyz,0)\) are obtained. From these distributions, structural and mechanistic information may be deduced.

Depending on the unit cell dimensions of the crystal, its limiting crystallographic resolution and the complexity of the structural mechanism being examined, a complete set of four-dimensional, time-resolved data may contain of order 10 000 unique values of \((hkl)\) and 100 values of \(t\). Laser pulses in the ns to 100 fs range have been used as the pump, and unchirped synchrotron X-ray pulses of around 100 ps duration as the probe. The time resolution \(\Delta t\) achieved (eqn. (1)) ranges from a few ns, determined largely by the duration of the pump laser pulse \(\Delta t_L\), to a few hundred ps.
determined by the duration of the probe X-ray pulse $\Delta t_Z$ and the jitter in the delay time $\Delta t_J$. Sources such as the ID9 beamline at ESRF or 14 ID-B at BioCARS/APS deliver around $10^8$ photons per 0.1% bandpass per X-ray pulse through a 200 µm aperture to the crystal, with a duration of around 100 ps.

The limitations on pump–probe experiments can be addressed if an energy-chirped X-ray probe pulse replaces the unchirped pulse. Suppose that a simple, perfectly energy-chirped X-ray pulse of 100 ps total duration (Fig. 1a) falls on a crystal and generates a Laue diffraction pattern. Laue spots stimulated by the lowest energies near $E_{\text{min}}$ correspond to the earliest times of arrival of the X-ray pulse at the crystal, and those stimulated by the highest energies near $E_{\text{max}}$ to the latest times of arrival. Suppose further that the pump laser pulse occurs during the probe X-ray pulse, at a time when the incident X-ray energy is $E$. The structure amplitudes derived from all Laue spots stimulated by energy $E_{\text{spot}}$ where $E_{\text{min}} < E_{\text{spot}} < E$ arise from the unperturbed structure and yield $|F(hkl,0)|$. Each spot where $E < E_{\text{spot}} < E_{\text{max}}$ arises from a perturbed structure and yields $|F(hkl,t_{\text{spot}})|$ where $t_{\text{spot}}$ is the time after the laser pulse that corresponds to $E_{\text{spot}}$ (Fig. 3). Thus, each Laue image automatically contains the internal control $|F(hkl,0)|$ for certain values of $(hkl)$ and a set of time-resolved data $|F(hkl,t)|$ for a range of values of $t$, for other values of $(hkl)$. This time range is set by the total duration of the chirped pulse and by the time of arrival of the laser pulse. No time-resolved X-ray detector is required. The experimental diffraction pattern itself contains information on the delay time between the laser and X-ray pulses; jitter in the delay time is irrelevant. Finally, suppose that the energy-chirped X-ray pulse contains the same number of photons per 0.1% bandpass as the original, unchirped pulse; the photons are simply redistributed in time within the pulse in a more experimentally-convenient manner. Hence, we see that the intensity of each Laue spot, the background underlying the spots and the exposure time per diffraction image are unaltered in replacing the unchirped by the chirped pulse.

The Laue diffraction pattern obtained from one crystal orientation contains only a subset of values of $(hkl)$. If the experiment is repeated at a set of slightly different orientations of the crystal, then complete coverage of the unique volume in reciprocal space can be achieved. As the crystal is re-oriented, each spot samples a different energy $E_{\text{spot}}$ corresponding to a different value of $t_{\text{spot}}$ and yields $|F(hkl,t_{\text{spot}})|$. If the total duration of the chirped pulse does not cover the complete time range needed, then the time of arrival of the laser pulse can be adjusted to precede all of the chirped X-ray pulse. In this case, no automatic measurement of $|F(hkl,0)|$ occurs.

![Fig. 3](image-url) Schematic of a simple energy-chirped pulse, in which a laser pump pulse occurs during the X-ray pulse when the X-ray energy is $E$. All spots that arise from energies between $E_{\text{min}}$ and $E$ diffract before the laser pulse; all spots that arise from energies between $E$ and $E_{\text{max}}$ diffract after the laser pulse. In particular, a spot with energy $E_{\text{spot}}$ diffracts at a time $t_{\text{spot}}$ after the laser pulse.

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A similar experiment is possible if a spatially-chirped X-ray pulse replaces the unchirped pulse. When a nearly parallel, monochromatic X-ray beam is incident on a stationary crystal, a “Laue still” diffraction pattern is generated that contains only those spots \((hkl)\) lying on the corresponding Ewald sphere. As the direction of propagation of the incident beam changes, the Ewald sphere also changes and gives rise to a new set of spots. In a spatially-chirped pulse, each direction of propagation (that is, each incident ray) corresponds to a different time of arrival of the probe pulse and all rays together constitute a fan (Fig. 1c). If the pump laser pulse arrives at a time corresponding to one particular ray in the fan, all earlier rays generate spots from which values of \(|F(hkl,0)|\) may be obtained. Each later ray corresponds to a known value of the time delay \(t\) and generates spots that yield \(|F(hkl,t)|\).

There are disadvantages to the use of a spatially-chirped pulse. The spots on the Laue still generated by each ray are displaced relative to their neighbors; the spots derived from the fan are elongated. Although each spot retains the same number of photons, the peak height of each and the signal to noise will be diminished. The possibility of overlap between adjacent spots with different values of \((hkl)\) is increased. The properties of each ray (if they are discrete) or of the fan of rays as a whole (if they are continuously distributed in angle of incidence) must be such as to generate the integrated intensities necessary to yield accurate structure amplitudes.

In effect, a chirped pulse allows the four-dimensional space spanned by \((hkl)\) and \(t\) to be scanned in an unusual way: an individual Laue diffraction pattern contains spots that arise from both a subset of the values of \((hkl)\) and a range of values of \(t\), notably including 0. This should be contrasted with the two normal ways of spanning this space. In the first (as for example in ref. 4 and 5), \(t\) is kept fixed and data are collected at a series of orientations of the crystal to cover all of \((hkl)\); then \(t\) is altered and the process repeated (if necessary with new crystals), until all desired values of \(t\) are covered. Data at \(t = 0\) are obtained in a separate experiment. In the second (S. Anderson et al., unpublished results), the crystal is kept in a fixed orientation and data are collected over all desired values of \(t\) including 0; then the crystal orientation is altered and the process repeated (if necessary with new crystals) until all unique \((hkl)\) values are covered. The systematic errors that affect data acquisition and reduction in both these ways should be greatly minimized by the use of chirped pulses, in which the \((hkl)\) and \(t\) variables are intermingled.

(c) Application of chirped pulses to other energy-dispersive experiments

An effective strategy for an X-ray absorption experiment is to conduct it in energy-dispersive mode. The beamline optics deliver a polychromatic X-ray beam to the sample that is highly collimated in one angle dimension but strongly convergent in the other. In the fan of X-rays falling on the sample, each ray corresponds to a different X-ray energy. If an energy-chirped X-ray pulse is used, each spatial location on the detector corresponds to a different time of arrival at the sample. A time-resolved X-ray absorption experiment can then be carried out in the same manner as the Laue experiment:
lower X-ray energies correspond to earlier times of arrival of the probe, X-ray pulse relative to the pump, laser pulse, and higher energies to later times. To obtain complete coverage of the energy- and time-dependent absorption spectrum $\gamma(E,t)$, one strategy is to vary the time of arrival of the laser pulse relative to the X-ray pulse. Alternatively, the X-ray optics may be adjusted to alter the relation between energy and angle of incidence on the sample.

In energy-dispersive powder diffraction, a point detector with excellent energy resolution is positioned at a fixed scattering angle with respect to the polychromatic, highly collimated, incident X-ray beam. If an energy-chirped X-ray pulse is used, the energy resolution automatically maps into time resolution. To obtain the complete set of $I(E,t)$ values, variation of the time of arrival of the laser pulse relative to the X-ray pulse will be necessary.

**Generation of chirped X-ray pulses**

(a) **Introduction**

The potential applications of chirped X-ray pulses have not been widely considered because until very recently, there was no suitable source of such pulses. In a conventional synchrotron source, the radiation from each electron as it traverses a bending magnet, wiggler or undulator is independent of that from all other electrons in the bunch and in particular does not depend on longitudinal position in the bunch. The pulse of radiation from the bunch is therefore unchirped. 17

Two novel methods have been proposed of manipulating an individual particle bunch in a synchrotron with the goal of obtaining a pulse of X-rays short with respect to the normal bunch length of 50–100 ps. Both methods also produce a chirped X-ray pulse, though that was not their explicit object. The first28 employs an unusual RF cavity that in effect turns the bunch on its side; it has not been tested experimentally. The second29,30 modulates the electron energy of a small slice of the electrons in a bunch as they pass through an undulator, by interaction with a fs laser pulse that co-propagates with the electron beam. Electrons of different energies pass through an immediately downstream dispersive element, which separates them in space according to their energies. Since electrons of different energy traverse different path lengths, they are also separated in time. That is, the source exhibits both an energy and a spatial chirp. Initial experiments confirm that this method works.30 A limiting feature is that only a small fraction of the electrons in a synchrotron bunch are affected, and hence only a small fraction of the total number of X-rays that would be emitted by the electron bunch are chirped.

Chirped pulses in the UV–visible region of the spectrum are obtained by passing an unchirped, polychromatic pulse over a grating pair in which photons of different energies traverse different path lengths.31 Equivalent schemes in the hard X-ray region of the spectrum would utilize, for example, multilayer pairs or asymmetric-cut crystals.28,32 Indeed, all X-ray dispersive elements automatically introduce an X-ray chirp, but its magnitude is in general extremely small. Hard X-ray delay lines can therefore be envisaged. However, all such schemes are subject to Liouville’s Theorem on the conservation of phase space volume. If a chirped pulse is derived from an initially unchirped pulse, it is expanded in time; and conversely, a lengthy, chirped pulse can be compressed into a brief, unchirped pulse. Thus, application of a hard X-ray delay line to an unchirped, 100 ps synchrotron X-ray pulse would result in a chirped pulse of much longer duration, say 1 ns. Further, the time resolution can never be less than the original pulse length, here 100 ps. It cannot approach the desired fs time range.

(b) **The sub-picosecond photon source (SPPS)**

The experimental situation has been transformed by initial conceptual and experimental steps towards the LCLS. A precursor known as the SPPS has very recently been proposed33 that does not depend on the SASE principle but is designed to generate very brief X-ray pulses of under 100 fs. The SPPS is based on modifications to the existing SLAC linac. Briefly, the SPPS exploits the fact that if the low emittance requirements essential to the SASE principle are relaxed, then it is possible to substantially compress the electron beam in longitudinal phase space (that is, in time) without excessive blow-up in the transverse phase space (that is, in position and momentum). These
compressed, high energy electron bunches then generate very short X-ray pulses as they pass through a suitable undulator. The full SPPS scheme proposes three stages of compression of the electron bunches as they traverse the SLAC linac (Fig. 5): a damping ring to linac compressor and bends (electron energy at the exit 1.19 GeV, bunch length $\sigma_z$ around 1.16 mm i.e. 3.86 ps), a new chicane compressor (9 to 10 GeV, around 50 $\mu$m, 165 fs) and a second new chicane compressor at the entrance to the Final Focus Test Beam (FFTB) facility (28 GeV, around 26 $\mu$m, 86 fs). The final result would be an almost entirely unchirped electron bunch of 80 fs FWHM duration, with an energy spread $\sigma_E/E_{el}$ of 1.5% around its mean of 28 GeV and a peak current of 30 kA. If this electron bunch then passes through a 10 m undulator, unchirped hard X-ray pulses of less than 100 fs duration and an X-ray flux of around $3 \times 10^7$ photons per 0.1% bandwidth per pulse would be produced. This value is nearly comparable with that delivered by ID9 at ESRF and 14 ID-B at BioCARS/APS of around $10^8$, used successfully for our time-resolved experiments. That is, this form of the SPPS would deliver polychromatic X-ray pulses comparable to those available today from the ESRF and APS undulators, but in a pulse that is three orders of magnitude shorter in time.

These 100 fs SPPS pulses could, in principle, be used directly for pump-probe single crystal X-ray diffraction experiments, exactly as with the present 100 ps APS pulses. However, jitter in the relative timing of the fs pump laser pulses and the 100 fs X-ray pulses becomes critical to the time resolution of the entire experiment, as discussed above; any structural changes on this very short time scale are likely to be very small and prone to being masked by systematic and random error in the time-dependent X-ray structure amplitudes; and if non-linear effects turn out to be significant,
the 100 fs X-ray pulses may be more damaging to the crystals than otherwise comparable 100 ps pulses.

These limitations can be addressed by a modification to the full SPPS scheme that utilizes the less-compressed, chirped electron bunches at lower energy. Note that the electron bunch immediately prior to the new 9 GeV chicane is highly chirped, with an energy spread of 1.7% or 154 MeV. The chirp is nearly linear, and the bunch length $\sigma_z$ is 1.16 mm or 3.86 ps. The full electron energy range of $-3\sigma_E$ to $+3\sigma_E$ then spans 8.54 GeV to 9.46 GeV, and a full duration of 23.2 ps (Fig. 6A). Suppose that this electron bunch is delivered to an undulator whose specifications are those of an APS undulator A with 100 poles and a period of 3.3 cm, operated at $K = 0.5$. (These specifications are used simply for definiteness; the optimum insertion device for this source is likely to have rather different parameters). The X-ray photon energy $E_{\text{photon}}$ of the $n$-th harmonic is given by:

$$E_{\text{photon}} = \frac{0.95nE_{el}^2}{(1 + K^2/2)\lambda_u}$$

and

$$dE_{\text{photon}} = \frac{1.9nE_{el}dE_{el}}{(1 + K^2/2)\lambda_u}$$

Fig. 6 A. Schematic of the profile of an energy-chirped electron pulse at 9.00 GeV with $\sigma_E = 154$ MeV. The $\pm 3\sigma_E$ energies are noted. B. Schematic of the profile of the energy-chirped X-ray pulse obtained by passing the electron pulse in A through an undulator (see text). At each point, the X-ray energy spans a small spectral range of width $\Delta E$, shown schematically in the inset.
$E_{\text{photon}}$ thus ranges for the first harmonic from 19.0 KeV at the leading edge of the pulse (lowest electron energy) to 23.3 KeV at the trailing edge (highest electron energy), in an X-ray pulse of around 23.2 ps full duration (Fig.6B).

Four main factors contribute to the time resolution available with such an X-ray pulse. First, the chirp in the electron bunch is not perfect; electrons of a given energy occupy a small range of longitudinal positions in the bunch, which corresponds to a small time range $\Delta t_{\text{chirp}}$. Second, the chirp and hence the mapping of energy into time may not be stable from pulse to pulse, and gives rise to $\Delta t_{\text{pulse}}$. This factor will not be significant if the zero time can be successfully extracted from each diffraction pattern, as suggested above. Third, electrons of a given energy generate an X-ray spectrum in the $n$th harmonic of width $\Delta E_{\text{photon}}$ where

$$\Delta E_{\text{photon}}/E_{\text{photon}} \sim 1/nN$$

where $N$ is the undulator period (Fig. 6B, inset). X-rays of a given energy thus originate from electrons of a small range of energy, and hence from a small range of time, $\Delta t_{\text{spectrum}}$. Fourth, indexing of the Laue diffraction pattern generated when such a chirped X-ray pulse falls on a single crystal assigns an energy $E_{\text{spot}}$ to each spot with an uncertainty $\Delta E_{\text{spot}}$, corresponding to $\Delta t_{\text{spot}}$. The overall time resolution is therefore given by

$$\Delta t_{\text{experiment}} = (\Delta t_{\text{chirp}}^2 + \Delta t_{\text{pulse}}^2 + \Delta t_{\text{spectrum}}^2 + \Delta t_{\text{spot}}^2)^{1/2}.$$  

Of these four factors, the third appears to be the most significant. In particular, $dE_{\text{photon}}/\Delta E_{\text{photon}} \sim 2nNEd_{e}/E_{el}$. The larger this quantity, the better the time resolution. This suggests that the largest electron energy chirp consistent with pulse stability, and a high harmonic $n$ derived from an undulator with many periods $N$, should be employed.

The above discussion has concentrated on the use of the chirped 9 GeV electron bunch. There is another possibility: pass the final, compressed, nearly unchirped, 28 GeV electron bunch through an expander, to generate a longer bunch with a suitable chirp prior to entering the undulator.

**Conclusions**

Achieving an experimental time resolution in the 100 fs range will be challenging. The conventional pump–probe experiment using an unchirped X-ray pulse will be limited by jitter in the relative timing of the two pulses; and the unconventional experiment proposed here using a chirped X-ray pulse will be limited by the characteristics of the X-ray chirp. Nevertheless, the effort will be worth while if important biological problems can be addressed.15

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