

Recent Advances in the Generation of Pulsed Synchrotron Radiation Suitable for Picosecond Time-Resolved X-ray Studies

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Abstract: We describe the beamline optics, the shutters and the femtosecond laser used in pump & probe experiments down to 50 picosecond resolution. The performance of in-vacuum undulators is compared to in-air undulators. It is concluded that single-harmonic in-vacuum undulators are optimal for pump and probe experiments based on single-bunch exposures. These low-K undulators can produce a spectral flux of 4.0×10^8 ph/0.1%bw per pulse at 15 keV, a factor of 5 higher than standard in-air undulators. The Laue diffraction study of the photolysis of CO in myoglobin is described. The study shows that high-level photolysis is possible with laser pulses as short as 100 fs and that the radiation damage from repeated x-ray and laser exposures is small. The success of the experiment indicates that the time-resolution of macro molecular crystallography may reach 200 fs with a future free electron laser (XFEL).

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1. Introduction

This article describes time-resolved structural studies of chemical and biochemical systems using pulsed synchrotron radiation from third generation synchrotrons. The pulsed radiation from an undulator at synchrotrons such as the European Synchrotron Facility (ESRF) in France, the Advanced Photon Source (APS) in the USA and SPring8 in Japan, is a new and important tool for time-resolved investigations of chemical and biochemical reactions. We will discuss how the pulse structure of can be used to probe short-lived intermediates along a reaction pathway in systems ranging from crystals (small molecules and macromolecules), liquids (molecules in solution and elemental liquids) to dilute gases.

The majority of fast experiments employ the pump and probe scheme: a short laser pulse initiates the molecules and a delayed x-ray pulse probes the outcome at a given delay. The laser pulse raises the energy above an initiation barrier and synchronises the initiation. While the “before” and “after” structures are known for most reactions, the pathways linking these limits are largely unknown and, till now, largely unexplored. The ability to watch the structural evolution of macromolecules by a sequence of static data sets, each

representing a snapshot at a given time in the reaction scheme, was developed at the ESRF in 1995 [1, 2] and is now actively pursued at APS and SPring8.

The success of fast time-resolved experiments has required several critical advances. The sample must be illuminated by an intense x-ray pulse from an undulator and delivered to the sample via suitable x-ray optics. The time-structure and duration of the x-ray pulse must be controlled both by the mode of operation of the storage ring and by a fast shutter train; the sample must be stimulated, uniformly and rapidly, by an intense laser pulse, to achieve reaction initiation; the time delay between the arrival of the laser pulse – the pump – and the x-ray pulse – the probe – must be suitably adjustable; and the x-rays that have interacted with the sample must be collected on a sensitive detector. In addition, the sample itself must be robust, to permit the accumulation of the numerous individual measurements that comprise a complete data set. That is, the sample must withstand illumination by numerous x-ray and laser pulses without exhibiting significant radiation damage.

We consider these technical advances and illustrate their application to time-resolved macromolecular crystallography and diffuse scattering from liquids. Our discussion is limited to the ESRF, but we emphasise that similar experiments may be conducted at other sources, including new high brilliance sources such as the Swiss Light Source. For a recent review of synchrotron technology and research, the reader is referred to [3].

2. Pulse structure of synchrotron light

The ESRF synchrotron consists of three synchronised accelerators: a 200 m long linear accelerator (linac) operated at 200 MeV; a 300 m long booster synchrotron which accelerates the electrons to 6 GeV; and an 844.1 m storage ring. It takes 2.82 μs for an electron to complete one turn in the storage ring. The energy lost to synchrotron radiation is compensated by an accelerating radio frequency field (RF), which operates at the 992nd harmonic of the orbit frequency. The machine physicists can now selectively inject electrons into these 992 bunch positions and the main bunch patterns are shown in figure 1.

The filling of the storage ring is obtained in the following way. A 1.0 μs electron pulse is accelerated in the linac to 200 MeV and then sent into the booster which is set for a closed orbit at 200 MeV. The energy of the electrons is then raised to 6 GeV by the RF while the magnetic field is increased to maintain a closed orbit. The RF field compresses the electrons into 352 bunches at the phase stable point: the leading electrons in the 30 mm long bunch arrive while the RF field is increasing, causing the electrons in the trailing part of the bunch to experience a slightly stronger field, thereby compressing the bunch. The bunches are now let into the storage ring via a transfer line. The booster is then refilled and the bunches accumulated in the storage ring at a frequency of 1 Hz. The filling of the storage ring takes typically 2-3 min for multi-bunch modes. The 1-bunch and 16-bunch mode are produced by a 1.8 ns electron pulse, which is compressed by the RF into a 100 ps pulse in the booster. The bunches are gradually accumulated in the storage ring. The accumulation of these modes takes longer due their higher current per bunch and the bunch cleaning which is necessary in order to suppress neighbouring bunches, which may be populated due to the tails in the initial 1.8 ns pulse. The filling procedure takes 10-15 minutes and the pulse purity may be as good as 1×10^{-7} .

The different bunch patterns differ in total current, current per bunch, lifetime and dead time between bunches. Different filling patterns optimise different experiments, and some patterns may be unsuitable for some experiments. For example, many experiments require high current and long lifetime, incompatible with single-bunch mode. Conversely, the most demanding time-resolved experiments require single-bunch and the experiment must tolerate lower total current and reduced lifetime. The pulse length depends only on the

total charge in the bunch. It varies between 50 ps in multi-bunch to 200 ps in single-bunch mode, see figure 2.

The lifetime of the beam is a function of the vacuum in the storage ring, the energy acceptance of the machine and the charge in the bunch. In multibunch mode, the lifetime is determined by the cross section of the elastic scattering (Rutherford) and inelastic scattering (bremsstrahlung) against residual molecules in the vacuum [4]. The lifetime is ca 55 hours at 200mA. The lifetime in single-bunch mode, on the other hand, is dominated by Toucek scattering, i.e. electric repulsion within the bunch. The lifetime is typically 6 h at 16 mA. The reduced lifetime may be of less experimental consequence if more frequent refilling of the storage ring is possible and indeed the possibility of continuous injection into the storage ring is being investigated. Note that the fill patterns differ markedly in the length of the “dark” periods between bunches, which is a key parameter for the x-ray chopper described below.

The most frequently used operation mode at the ESRF is the 2/3 filling, which has 660 bunches placed side by side 2/3 of the ring. The APS has chosen a 75-bunch mode comprising groups of 25 triplets, which are uniformly distributed around the ring. Finally, SPring8 uses 2016 bunches in 24/29 of the ring. It should be emphasised that these bunch patterns are undergoing a continuous development and that they may change in the future with the aim of producing higher currents and longer lifetimes. The machine parameters of the ESRF, APS and Spring8 are shown in table 1.

3. Insertion devices for high-flux x-ray generation

The characteristics of the undulator source, i.e. its brilliance (ph/0.1%bw/mm²/m²/bunch), its flux (ph/s/0.1%bw/bunch), its tunability and bandwidth, can within certain limits, be optimised for specific experiments. Single bunch diffraction experiments require high flux, small angle scattering a parallel beam and x-ray spectroscopy energy tunability. For single-bunch diffraction, the figure of merit is the spectral flux in the focal spot, which can be collected in one pulse. That can be calculated considering the magnetic and geometric properties of the undulator, the properties of the optical elements (including their imperfection and thermal deformation) and losses in windows and detectors. In the following we will calculate the maximum number of photons that can be produced by a 2.0 m long undulator during the passage of one bunch. We will briefly review the properties of undulators and see how they are influenced by the magnet technology and the minimum gap.

An insertion device consists of an array of alternating magnets, which forces the electrons into a sinusoidal motion about the incident direction [5]. When the electron beam is sent through a vertically oscillating field of wavelength λ_0 ,

$$B_z = B_0 \sin\left(2\pi \frac{x}{\lambda_0}\right),$$

the tangent to the orbit (or angular deflection) is:

$$\beta_y(x) = -\frac{K}{\gamma} \cos\left(2\pi \frac{x}{\lambda_0}\right),$$

where γ is the relativistic parameter $(1-\{v/c\}^2)^{-1/2}$, $\gamma = 1957 \text{ E(GeV)}$. The deflection parameter K is defined as

$$K = \frac{e B_0 \lambda_0}{2\pi m c} = 0.934 B_0(T) \lambda_0(cm),$$

where e and m refer to the electron charge and mass. Note that the tangents fall within a cone of opening angle $\pm K/\gamma$ around the forward direction. In the curved part of the orbit, the electron emits polychromatic radiation, which moves forward at the speed of light. The electron, which moves nearly at the speed of light, follows the longer sinusoidal trajectory imposed by the magnetic field. The frequencies that oscillate in phase with the reappearance of the electron interfere constructively, leading to amplification of the radiation. The odd harmonics are amplified and the even harmonics vanish due to the opposite directed electric fields from the 2nd, 4th etc harmonic. The wavelengths are determined by:

$$\lambda_n = \frac{1 + K^2/2 + (\gamma \theta)^2 + (\gamma \varphi)^2}{2 n \gamma^2},$$

where θ and φ are the off-axis angles in the horizontal and vertical directions respectively. Numerically,

$$\lambda_n \text{ (A)} = 13.056 \lambda_0 \text{ (cm)} \frac{1 + K^2/2 + (\gamma \theta)^2 + (\gamma \varphi)^2}{n E^2 \text{ (GeV)}},$$

with the corresponding energy

$$\varepsilon_n \text{ (keV)} = 0.95 \frac{n E^2 \text{ (GeV)}}{\lambda_0 \text{ (cm)} \{1 + K^2/2 + (\gamma \theta)^2 + (\gamma \varphi)^2\}}$$

The on-axis spectrum of a single electron is the sum of the odd harmonics. When the size of the aperture increases (finite values of θ and φ), the spectrum is broadened towards lower energies. The result is a triangular energy band with a cut-off given by the on-axis energy. In a real synchrotron, the energy and angular dispersion of the electron beam broaden the bandwidth further. The spectrum of an undulator with 235-poles on a 17 mm period and a K of 0.86 is shown in figure 3.

The angular divergence of the radiation from the n th harmonic from a single electron is concentrated in a cone about the forward direction with an RMS width of:

$$\sigma_r = \frac{1}{\gamma} \sqrt{\frac{(1 + K^2/2)}{2N n}},$$

where N is the number of periods. Note that the opening angle is proportional to $1/\sqrt{N}$. The value of σ_r is 6.5 μ rad for the U17 fundamental at 14.8 keV. The divergence of the photon beam is now the convolution of σ_r with the electron divergence. At the ESRF, the electron beam is focused in the centre of the undulator straight sections into so-called low- β and high- β sites. The electron focusing is shown in table 2. Note that the two sites are identical in the vertical. Here the electron divergence is 3.9 μ rad and the single-electron photon divergence 6.5 μ rad. That produces a vertical divergence of 7.6 μ rad for both sites. In the horizontal, the electron divergence is 87.8 μ rad and 12.3 μ rad on the low- β and high- β site respectively, which determines the photon divergence of the undulator. The flux through a small pinhole placed far from the source, is higher for high- β sites due to their lower horizontal divergence. The high- β sites are advantageous for experiments without focusing optics and parallel beam experiments. The high- β source is elongated with

RMS values of 391.7 μm and 9.8 μm . The low- β site is small with RMS values of 56.5 μm and 10.2 μm . The horizontal divergence is larger, which increases the size of the optical elements. The low- β site is the best choice for time-resolved diffraction provided that the focusing optics can accept the full horizontal size of the beam.

The flux on the n^{th} harmonic of an undulator produced by a filament beam is:

$$I_n(\text{ph/s}/0.1\%bw) = 1.432 \times 10^{14} N I(A) Q_n(K),$$

where $I(A)$ is the current in Amperes and

$$Q_n(K) = (1 + K^2/2) F_n(K) / n,$$

where

$$F_n(K) = \frac{n^2 K^2}{(1 + K^2/2)^2} \left\{ J_{(n-1)/2} \left(\frac{n K^2}{4(1 + K^2/2)} \right) - J_{(n+1)/2} \left(\frac{n K^2}{4(1 + K^2/2)} \right) \right\}^2.$$

$J_n(x)$ is a Bessel function of the first kind. These functions are shown in figure 4 and figure 5. Note that I_n is linear in N but independent of E . It is seen from figure 4 that the spectrum is dominated by the first harmonic when $K < 0.5$. Such undulators have limited tunability but their heat load is low. For $K > 0.5$, higher harmonics are stimulated due to the increasing hardness of the bending magnet spectra along the orbit. The tunability and heat load increase with increasing K . Note that the amplitude of $I_1(K)$ reaches 80% of its saturation value at $K=1.5$. From a flux point of view, there is no interest in having a high- K undulator (provided that the fundamental energy can be obtained by a low- K device).

The central brightness of the n^{th} harmonic from a filament beam is:

$$\frac{d^2 I_n(0, 0)}{d\theta d\phi} (\text{ph/s}/0.1\%bw/mr^2) = 1.774 \times 10^{14} N^2 E^2(\text{GeV}) I(A) F_n,$$

which is linear in N^2 and E^2 . The E^2 dependence comes from the $1/\gamma$ angular scaling and N^2 from the angular reduction of the central cone with the number of periods. Note that each harmonic has a maximum at a given K . For example, the optimal brightness of a single harmonic undulator is $K = 1.20$.

The above discussion is only valid for a fixed number of periods. When we impose a finite undulator length, gap and photon energy, the properties of the magnetic array has to be considered. We will calculate the optimal flux per pulse below. The magnetic field in the insertion device is normally provided by permanent magnets such as NdFeB or SmCo, which produce fields up to 2 Tesla. In practice, one has to strike a compromise between the number of periods and the K -value: as the period becomes shorter, the field from neighbouring poles begins to reduce the amplitude of the field. The sinusoidal amplitude of an array of dipoles is described by the Halbach equation [1]:

$$B_0 = B_1 \exp\left(-\pi \frac{g}{\lambda_0}\right),$$

where B_1 is the saturation field and g is the gap between the magnets. B_1 depends on the magnetic material. The latest undulators at ESRF have their magnets in vacuum. These magnets are made of SmCo with $B_1 =$

1.65 T [2, 3]. Recent tests have shown that the gap can be lowered to 6.0 mm without any reduction in the lifetime of the electron beam. For a given value of E_1 , B_1 and g_{\min} , λ_m and K are determined from the Halbach equation. The result is shown in figure 6. The total length of 2.0 m determines then the number of poles and the flux on the harmonics. The properties of undulators with fundamentals at 10, 15, 20 and 30 keV are listed in table 3. The undulators produce from 1×10^8 to 4×10^8 ph/0.1%bw for a 16 mA bunch in the energy range 10-30 keV. This is probably the limit for a 2.0 m undulator source with conventional non-superconducting magnets, since higher currents per bunch and smaller gaps will reduce the lifetime of the beam. It is seen from table 3, that a 30 keV beam can either be obtained from the first harmonic of a low-K device (U11.0), or as the third harmonic from medium-K device (U20.1). The low-K device will, however, produce a low-divergence and low-power beam of superior flux, but of very limited tunability. For these reasons most multipurpose beamlines have a low-K ($K < 1$) and medium-K ($1.5 < K < 2.5$) device.

The standard gap of an in-air undulator is 16 mm and the gain in flux in going to 6 mm is shown in figure 7. The gain at 15 keV, the optimal energy for conventional protein crystallography, is 5!

The ID09 beamline will have a new U17 in-vacuum insertion device installed during the summer 2001. The present insertion devices are listed in table 4 and their spectra shown in figure 8. The figure shows the focused flux from the U20 undulator and W70 wiggler. The beams are focused by a platinum coated toroidal mirror receiving a 10 mmh x 1.4 mmv beam 30 m from the source. The incidence angle is 2.335 mrad and the energy cut-off 38 keV. The monochromatic flux from a silicon (111) monochromator is calculated by applying the relative bandwidth 1.4×10^{-4} . The y-axis on the left in figure 8 shows the intensity from a 180 ps pulse from a 16 mA bunch. Note the low flux below 7 keV, which is due to absorption in the vacuum windows in the beamline (1.0 mm beryllium and 0.26 mm graphite).

4. X-ray Optics for picosecond experiments

The ID09 beamline has optics and detectors to perform pump and probe experiments in diffraction from macro- and small molecules, diffuse wide-angle scattering from liquids and surface melting experiments with a streak camera. Single pulses of x-ray are selected by a chopper, which runs synchronously with a femtosecond laser at 900 Hz. The x-ray optics is shown in figure 9. The beamline can be configured in both mono- and polychromatic modes. The polychromatic mode for Laue diffraction is particularly difficult due to the heat load from the focused wiggler, which ruined the intrinsic stability of the electron source point. We have therefore installed a 20-ms shutter upstream the toroidal mirror to relieve all down stream elements from the heatload from a focused wiggler.

4.1. Monochromator

The ID09 beamline has a water-cooled monochromator designed to operate between 4.9-39.6 keV. The water-cooling can extract 25 W before the rocking curve starts to broaden. It is mainly used with the U20 undulator and occasionally with the U46 and W70 at low currents, i.e. single-bunch mode. The thermal slope-error of the first crystal was studied for different cooling geometries in order to optimise the throughput of the two crystals. The optimal crystal geometry was found to have a U-shaped cross-section with the beam-footprint at the bottom of the U and cooling contacts at the top. That constrains the heat to flow laterally and minimises the in-depth thermal gradient, which is responsible for the bending in the scattering plane.

The distance between the two crystals is held fixed at $\rho = 10.0$ mm, and that causes a vertical shift in the position of the beam with Bragg angle ($\delta h = \rho \sin 2\theta / \sin \theta$). In energy scans the height of the experimental table is linked to the energy of the monochromator such that fixed-exit scans can be realised. The crystals

are mounted in a holder that has a weak mechanical link that allows adjusting the angle between the crystals. The crystal holder is fixed onto a rotation table, which is driven from outside the vacuum. The horizontal beam position is controlled at two levels: a stepper motor tilts the first crystal and a piezo tilts the second with high resolution.

The rejection of harmonics has to be considered due to the fixed energy cut-off at 38 keV of the toroidal mirror. In the range 4.5-12.7 keV, the (333) reflection contaminates the beam. The problem can be solved by opening the gap of the undulator hereby the content of hard radiation is reduced.

The U17 undulator foreseen for ID09 in 2001, will produce a total power up to 2740 W and a power density up to 110 W/mm². Final element analyses has shown that at these power densities, the deviation from crystal planarity becomes close to the rocking width of Si(111) even at cryogenic temperatures(15 µrad at 15 keV). The monochromatic performance is summarised in table 5.

4.2. Focusing mirrors

The white beam is focused by a platinum coated cylindrical mirror, which is bend vertically into a toroid (and is therefore called a toroidal mirror). The mirror is 1.0 m long and placed 29.7 m from the centre of the straight section. It focuses the white beam at a glancing angle of 2.335 mrad. The second mirror is a 1.2-m long plane mirror, which is bent vertically into a parabola. The latter is used in micro-focusing experiments in high-pressure research, but it is also occasionally used for time-resolved experiments when a large and homogeneous beam is required. Both mirrors are water-cooled from the sides. The cooling system for the cylindrical mirror includes a copper roof, which is lowered into the cylindrical cut of the mirror. The roof absorbs the Compton scattering and shields the bending mechanism. The mirror parameters are summarised in table 6. The toroidal mirror was the first mirror received at the ESRF in 1992 (and a silicon mirror will soon replace it). The body of the mirror is made of graphite enclosed in a jacket of SiC. The slope-error in the central 380 mm of the mirror is 4.1 µrad(rms), substantially better than the overall average of 9.0 µrad(rms). In practice therefore, the intensity of the focus does not receive radiation from the outer parts of the mirror, a problem that is aggravated by strain from the mirror holder. The reflected power was measured and found to be 50 and 60% of the expected value for the toroid and parabolic mirror respectively. That indicates that the surfaces degrade gradually due to low-Z impurities from the vacuum and perhaps migration the binding substrate below the coating. The vacuum vessel for the toroidal mirror is pumped by two ion pumps and the pressure has now, after five years of pumping reached 1×10^7 torr. By contrast the vacuum in the second mirror vessel is 2×10^8 torr, which shows the superior vacuum compatibility of pure silicon.

The cooling of the mirrors works well for the single-harmonic undulator U20 where the focal spot can be stable to within ± 25 µm for days. This is due to the low power and the good matching between the undulator spectrum and the cut-off of the mirror. We have found that the long-term stability of the focal spot is determined almost exclusively by the heatload. The pointing stability of the focused wiggler was particularly difficult to handle. But given that Laue exposures are short and only repeated fairly infrequently (ca 1Hz), we decided to install a shutter in front of the toroidal mirror. It produces shots down to 20-ms. The shutter consists of a rotating tunnel in a water-cooled block of copper. The shutter is driven by a stepper motor and the water flows through the rotation axis. It can produce shots at repeats up to 2 Hz. The shutter has solved the drift problem and given more time for experiments.

5. Single X-ray pulse selection

The chopper consists of a triangular rotor made of titanium which has a channel carved into one of its sides, see figure 10. The channel is covered at the tips by small tungsten plates, which produce an entrance and exit slit in the channel. The chopper is installed in the beamline vacuum just before the sample in order to take advantage of the small vertical beam near the focus. In white beam experiments, the rotating shutter described above provides critical protection from the heatload from the focused beam. The chopper dimensions and performance are shown in table 7. The triangular shape is a compromise between high tip-speed and low inertia(safety). The rotor is held in magnetic bearings and rotates normally at 896.6 Hz, the 396th sub-harmonic of the orbit frequency. The speed is controlled by a feed back system which stabilises the rotor to a relative accuracy $\delta\omega/\omega$ of 1×10^{-5} . The speed at the tip is 545.3 m/s thus exceeding the speed of sound in air. Consequently the rotor has to run in vacuum. The shaft of the chopper exhibits a resonance at 998 Hz and the centrifugal breakdown frequency is 1300 Hz. It is thus impossible to obtain shorter opening times by increasing the frequency with this design.

The acceptance profile of the rotor is trapezoidal. Its base-line τ and opening profile $p(t)$ are:

$$\tau = \frac{a}{2 \pi R f \sqrt{1 - (h/R)^2}}$$

and

$$p(t) = \begin{cases} 0 & \text{if } Abs[\theta(t)] \geq \theta_{min} \\ Min\left[1, \frac{2R}{S} \sin(\theta_{min} - \theta(t))\right] & \text{if } Abs[\theta(t)] \leq \theta_{min} \end{cases}$$

with

$$\theta_{min} = Arc\ sin(a/2R) \quad \text{and} \quad \theta(t) = 2 \pi f t ,$$

where a is the height of the tunnel, R the maximum radius, h is the distance of the tunnel to the centre, f the rotation frequency and S the beam size. The base-line opening time can be calculated using the following parameters: $a = 0.7$ mm, $R = 96.8$ mm, $h = 47.35$ mm, $f_{max} = 896.64$ Hz, which gives $t_{min} = 1.472$ μ s. The first rotor, designed for single bunch extraction from the single-bunch and hybrid mode, had tunnel dimensions of 3.0 mmh x 0.7 mmv. In order to use the chopper in the more frequently used 16-bunch mode, we installed a new rotor with a trapezoidal cross section. The new tunnel is 4.0 mmh wide and its height increases linearly from 0.05 to 0.90 mm. The opening time can be varied from 0.10 to 1.89 μ s by translating the chopper laterally. The new tunnel design allows in principle to reduce the opening time to 0.105 μ s, well below the 0.352 μ s needed for the 16-bunch mode. The new design makes it possible to extract single bunches from the 16-bunch mode, which requires an opening window of at least 0.352 μ s.

The rotor chamber is a 40 mm thick steel cylinder which provides protection against rotor breakdown. The cylindrical chamber is held in a frame, which allows the rotor & chamber to rotate in case of transient transfer of energy from the rotor. The chopper is mounted in a position & tilt alignment stage, which permits centring the chopper precisely on the x-ray beam. When the chopper is stopped, the controller automatically drives into the open position. In fact in static experiments, the beam is always shot through the chopper tunnel. A PC controls the chopper commands. The phase jitter is displayed every two minutes and is typically 10.5 ns (RMS) at full speed. The timing control of the chopper is based on an ECL signal at 44 MHz(RF/8) signal, the

phase of which can be scanned with the full resolution of the RF, i.e. 2.84 ns. Note that the chopper selects single bunches at 896.6 Hz which corresponds to a pulse on the sample every 1.11 ms. In order to do single-shot experiments, the chopper has to be preceded by a ms-shutter. We have therefore installed a ms-shutter in the beamline vacuum in front of the chopper. It consists of a 60 mm long bar with a trapezoidal tunnel along its length. The tunnel is 5 mm wide and its height increases linearly from 0.3 to 2.0 mm. The tunnel is centred on the axis of rotation, which gives two openings per turn. The bar is mounted on the axis of a stepper motor, which is mounted outside the vacuum via a ferrofluid feed-through. Normally this shutter is used in single-shot mode. In its closed position, the shutter tunnel is pointing down at -90 degrees. The shortest exposure times are obtained by accelerating the tunnel from -90 to $+90$ degree and opening times down to 0.2 ms have been obtained.

The layout of the shutters is shown in figure 11 and figure 12. In the direction of the x-ray beam, we have a wire monitor for position measurements, the ms-shutter, the chopper, sample slits, the I_0 monitor, the beryllium window, a transport pipe, a goniostat, a motorized beamstop and a (x, y, z) stage for a MARCCD (133 mm). Since some experiments need the laser beam very close to the x-ray beam (surface melting), the x-ray beam travels through a 300 mm long pipe which has a collimator tip at the end. The pipe can be flushed with helium gas to reduce the effect of air scattering near the sample. The synchronization of a single-shot Laue experiment with the femtosecond laser is shown in figure 13.

Finally we note that there are other ways of extracting single pulses from a synchrotron. A rotating crystal is described in [4], a rotating mirror in [5] and rotating scanner drum is described in [6].

6. The femtosecond laser system

The femtosecond laser is the main instrument for ultra fast initiation of photo-chemical reactions. It is phase locked to the x-rays and used for single-bunch Laue diffraction, stroboscopic experiments at 900 Hz (diffraction and wide-angle scattering) and to trigger a jitter-free femtosecond x-ray streak camera. The laser system consists of three stages, see figure 14. The first stage is a Kerr-effect mode-locked Ti: sapphire laser that is phase locked to the synchrotron RF/4 and designed to produce weak 100 fs pulses near 800 nm with a repetition frequency of 88 MHz. The second stage, a Ti: sapphire regenerative chirped pulse amplifier (CPA) operating at a repetition frequency of 900 Hz, boosts the energy of a single fs pulse to about 0.5 mJ. Before amplification, the pulse is stretched from 100 fs to 160 ps. The stretching is necessary because the high peak power of a 1 mJ, 1 mm^2 , 100 fs pulse (1 TW cm^{-2}) would cause non-linear absorption and damage to several optical elements in the amplifier, including the Ti: sapphire crystal and the pockels cell. After amplification, the stretched pulse is ejected from the amplifier, and compressed back down to 100 fs. The third stage employs non-linear methods to generate a variety of wavelengths. The methods include frequency doubling (400 nm), third harmonic generation (267 nm), and optical parametric generation/amplification (OPG/OPA). The OPA/OPG can generate tuneable visible pulses (460-760 nm) with up to $35 \mu\text{J}$ per pulse. The entire laser system is supported on a $4.25 \times 1.5 \text{ m}^2$ optical table.

From table 8 it is seen that a laser pulse contains more than 3×10^{13} photons which matches the number of binding sites in a $100 \times 100 \times 100 \mu\text{m}^3$ MbCO crystal. This pulse energy makes it possible, at least in principle, to produce a high degree of excitation. In practice one would like to have 5-10 times more photons to compensate for losses in the beamline between the laser and the sample. The laser is focused by a motorised telescope, which makes it possible to scan the laser focus onto the sample. The diffractometer and telescope are shown in figure 15.

7. Time-resolved experiments

The pump and probe set-up has been used in diffraction experiments on small and large molecules, in wide-angle scattering from liquids and in surface melting experiments. Historically the single-bunch Laue study of the photo-dissociation of CO in myoglobin was the first experiment to obtain three-dimensional pictures of a protein in action with 10 ns resolution. We will review this study and describe how the time-resolution has been pushed to 100 ps by the use of the femtosecond laser. In addition we have also performed single-bunch studies of an early intermediate in the photo-cycle of the yellow protein PYP, see [7].

7.1. Photo-dissociation of CO in Myoglobin

The first biological reaction that was studied was the dissociation and rebinding of CO in myoglobin (Mb). The study used single-bunch Laue diffraction and the photolysis was done using a 10 ns Nd: YAG laser, see [7, 8]. The biological function of myoglobin is to store oxygen in muscle cells, just as hemoglobin transports oxygen in blood cells. Muscle cells need myoglobin as peak-load buffer when the blood circulation cannot supply oxygen fast enough, for example during muscle contraction.

One would not normally refer to myoglobin as a photoactive protein. But one of its less known properties is that when exposed to an intense flash of light, it temporarily releases its oxygen. The structure of myoglobin is very similar to that of one of the four sub-units of hemoglobin, i.e. hemoglobin is a tetramer of four myoglobin units. Myoglobin is a compact globular molecule with a molecular weight of 17800 Dalton¹, and it consists of a single polypeptide chain of 153 amino acids.

The oxygen is bound by a Fe²⁺ atom in the centre of a porphyrin ring embedded within the hydrophobic interior of the protein. The porphyrin ring and its iron atom constitute the *heme* group, which is anchored to the protein through a covalent link to a proximal histidine. As figure 16 shows, the binding site is buried in the protein with no obvious opening to the outside. The motivation for doing time-resolved structural studies on myoglobin is to learn more about the pathway of the oxygen entering and escaping than could be guessed based on the static structure and to identify rapid structural changes associated with ligand release and rebinding. The distal histidine is supposed to act as a "door stop" with two stable positions, opening and closing a channel through which the ligand can enter or exit.

The solution of the atomic structures of myoglobin and hemoglobin by John Kendrew and Max Perutz respectively, were the first of many achievements in structural biology. By the end of the 1950s, this study was largely complete and demonstrated the power of X-ray crystallography. For practical reasons, Kendrew chose Sperm Whale myoglobin for his work because it is stable, crystallises well and was readily available.

The time-resolved studies are done on CO-ligated myoglobin MbCO rather than the natural oxy-myoglobin MbO₂. One of the reasons for this choice is the inherent instability of the latter. The oxygen tends to oxidise Fe²⁺ to Fe³⁺, which converts it to inactive met-myoglobin. Since the auto-oxidation constant for sperm whale myoglobin is 0.055 h⁻¹ at 37°C, see [9], a MbO₂ sample would have to be prepared on the day of the experiment. A more important reason for choosing CO over O₂ is the difference in their photolysis efficiency. In MbO₂ the photolysis is followed by rapid geminate recombination with most of the oxygen rebinding within 100 ps to its original iron atom. Although myoglobin binds CO more strongly than O₂, the rate of CO geminate recombination is far slower. Consequently, the photolysis yield for carbonmonoxy-myoglobin is nearly 100%.

¹ Atomic mass unit, approximately the mass of a hydrogen atom, 1 Da = 1 u = 1.66 · 10⁻²⁷ kg

Myoglobin binds CO 30 times more strongly than oxygen. This relative affinity ought to be compared to the relative affinities of pure *heme* in solution without the protein environment, where CO binds to the free heme 1500 times more strongly than oxygen. Clearly, the protein environment influences the relative binding affinities of CO and O₂. This relative inhibition is important: given this inhibition, CO produced endogenously by the metabolism of heme poisons only about 1% of the myoglobin.

Time-resolved infrared spectroscopy has provided evidence for an intermediate "docking site" for CO prior to its escape into solution [10-14]. The photo-dissociation is ultra-fast and completed in less than 1 ps. Then, the CO remains localised within a "B-state" docking site for about 200 ns after which it migrates through the protein and escapes into the surrounding solvent with a time constant of about 3 μ s. From there the rebinding is diffusion-controlled, and its time scale depends on the concentration.

In 1994 the existence of an intermediate site was identified by cryo-crystallography by Ilme Schlichting [15] and the group of Keith Moffat [16]. At temperatures below 40 K myoglobin loses its flexibility, so that after photolysis, the ligand can no longer escape from the *heme* pocket and remains trapped at a docking site. The results show clearly a docking site in the *heme* pocket with two possibilities for the orientation of the CO, together with a displacement of the iron atom out of the *heme* plane away from the CO (*heme doming*). However, it remains unclear whether the docking site found at low temperature is really the same as an intermediate state under physiological conditions.

For this reason we pursued a time-resolved crystallographic study of photolysed MbCO at room temperature where functional flexibility of the protein is retained. The time resolution of this study was limited by the laser pulse duration of 7 ns, which does not match the x-ray pulse length of ca 100 ps. For this reason the 7 ns laser was replaced with a 100 fs laser. The time resolution is thus only limited by the x-ray pulse length of ca 100 ps.

7.2. Femtosecond photolysis of MbCO crystals

The injection of light into a myoglobin crystal is a challenge due to the high optical density at optical wavelengths [17]. It is thus helpful to investigate the excitation efficiency prior to any experiment on a synchrotron. To that end, a 100 fs transient absorbance spectrometer, employing monochromatic excitation and polychromatic probing was designed alongside the fs laser system. The details will be published elsewhere. The spectrometer has micro-focusing capability, a must for optical studies of very small protein crystals. The principle of the spectrometer is shown in figure 17. A 300 μ m myoglobin crystal mounted in an X-ray capillary was excited with 100 fs pulses of 10 μ J at a wavelength of around 575 nm. The excitation beam was circularly polarised and focused to 200 μ m. The sample was mounted at an angle of 45° to the probe beam. The polarisation of the probe continuum was adjusted to find the minimum absorbance axis of the crystal. For this orientation, the peak absorbance of the sample was 0.7 at 540 nm and 0.3 at the photolysis wavelength of 575 nm. The spectra were averaged over hundreds of shots at each time-point to average out pulse to pulse fluctuations in the signal. After each time point, the dial on the translation stage of the delay line was advanced to set a new delay between the pump and the probe.

A series of transient absorption spectra are shown in figure 18. They show the development of the characteristic difference signature between deoxy and CO myoglobin shown in figure 19. After 340 fs it is almost fully developed and changes only slightly up to 10 ps. The transient spectra also reveal that there is no significant bleaching during the first 100 fs at any wavelength within the accessible range. If such a bleach would have been found, it would have been possible to enhance the penetration of the pulse by tuning the pump to that wavelength. From the relative amplitudes of the difference signal, the excitation was estimated

to 16%. Another conclusion from this experiment is that the MbCO crystal showed no sign of radiation damage even after absorbing thousands of intense 100 fs pulses.

7.3. Single bunch experiment on MbCO

In the Laue diffraction experiment, performed in single-bunch mode at the ESRF, the sample was photolysed by a 100-fs pulse of 22 μJ energy and 576 nm wavelength. This pulse energy was the maximum that could be obtained with the OPG/OPA system. The laser beam was circular polarised and focused to 200 μm FWHM at the sample. The sample was excited in a configuration in which the laser beam is perpendicular to the X-ray beam and collinear with the rotation axis of the diffractometer, see figure 20. The sealed capillary containing the sample is tilted 45° away from the rotation axis. The advantage is that the sample always exposes the same face towards the laser beam. The penetration depth and sample excitation degree does therefore not vary when the sample is rotated during the data collection. The circular polarisation ensures that the absorption is not affected by the dichroism of the crystal. The fact that the capillary is tilted 45° against the laser beam does not significantly increase reflection losses (2.8 % at normal incidence, 3.7 % at 45°). Due to space limitations near the sample, a long focal length of 1.5 m was needed to achieve a diffraction-limited focal point of 200 μm diameter and thereby match the sample size. An effective focal length of 1.5 m was obtained by setting up an optical telescope using a negative ($f = -175$ mm) and focusing ($f = +240$ mm) lens pair placed approximately 85 mm apart. The beam diameter at the first lens was about 8 mm and 12 mm at the second, giving a diffraction-limited focal spot diameter at the sample of about 180 μm . The experiment was performed with monoclinic crystals of CO-ligated myoglobin, prepared by Vukica Srajer at the University of Chicago. These crystals have a unit cell size of $a = 64.18$ Å, $b = 30.79$ Å, $c = 34.73$, $\alpha = \gamma = 90^\circ$, $\beta = 105.13^\circ$ and the space group symmetry $P2_1$. Relatively small crystals of about 200 μm diameter were used, which had an optical density (OD) of 1 at 576 nm.

The X-ray diffraction data was collected over a range of 180° in steps of 8°. For each setting of the crystal, 64 single-bunch shots were accumulated on the detector at a rate of 0.5 Hz. The laser heat load on the sample limited the accumulation rate to 0.5 Hz. The detector was an image intensified CCD camera, which was cooled by liquid nitrogen to eliminate dark current during the accumulation of the data on the CCD. To maximise the photon flux, two insertion devices were used in tandem: the W70(20.6 mm gap) and the U46 (16.3 mm gap). That produces a broad spectrum between 8 to 38 keV. To obtain a smoother spectrum, the gaps of both insertion devices were tapered 1 mm (difference in gap from input to output). A Laue pattern based on the accumulation of 10 single-bunch shots is shown in figure 21.

The data were processed using the *LaueView* software [18]. The new 100-ps data is being scrutinised at the time of writing and we will limit our discussion to the nanosecond maps shown in figure 22. These difference maps were calculated from 50000 structure factors analysed to 1.8 Å resolution. The reflections were measured with an average 5-fold redundancy and the data set was 98% complete. The map shows the difference in electron density of the MbCO molecule at five time points between 4 ns and 350 μs . The difference map is displayed relative to the protein backbone shown in yellow. Inside the red regions, the number of electrons decreases and inside the blue region it increases. The contour level of the maps is chosen at 3.5 σ of the peak electron density in the 4 ns map. Note that the density is gone in the 350 μs map, which shows CO takes 350 μs to recombine. A zoom of the 4 ns time-point is shown in figure 23. The positive peak below the heme plane is the heme doming: a movement of the iron atom out of the plane caused by the change in co-ordination from six to five when the Fe-C bond is broken. Above the heme plane, one finds three pockets of positive density. The first is placed next to the CO-hole and the second is placed

between the CO-hole and the Ile 107 residue. The third feature is near the His 64 residue and could be assigned to a relaxation of the His 64 “doorstop”, although the corresponding negative density is missing. The first pocket is potentially a docking-site. The second pocket coincides with that found in the cryo-structures. Its occupancy is small however. Note that if an atom moves from position A to B, the difference map will show a dipole distribution provided that the map is of sufficient resolution. Given that the His 64 residue is should move as a rigid body, the positive density just below may be noise. The excitation degree at 4 ns is estimated to be 40%. We believe that the future U17 undulator with its 10 fold increase in peak brilliance, its higher spectral purity combined with improved strategies for filtering of poor intensity measurements [19, 20] will increase the quality of difference maps from single bunch exposures.

The degree of excitation is a key parameter that deserves attention. It is readily shown that the signal-to-noise ratio of a difference map is proportional to the occupancy times the square root of the numbers of x-rays in the exposure. It is thus tempting to boost the excitation. Note that at an excitation level of 10%, a CO molecule shows up as 1.4 electrons. Such a signal level is close to the hydrogen level, which is normally neglected in protein crystallography! On the other hand, high-level photoexcitation increases the risk of two-photon absorption, which may lead to non-physiological states that contaminate the data. Additional effort to develop photolysis protocols that enhance the signal level while minimising multiphoton absorption and/or sample damage are needed. On the synchrotron side one needs a better single-line undulator with associated focusing optics. Combined with “smart” suppression of poorly measured reflections by using the known structure factors of the ground state, time resolved Laue diffraction is likely to become a powerful and useful method for structural investigations of biological molecules.

7.4. Photo-isomerization in the Photoactive Yellow Protein

The Photoactive Yellow Protein (PYP, also called *Xanthopsin*) is a photosensitive protein that has been isolated from a purple bacterium called *Ectothiorhodospira halophila*. This organism lives in salt lakes under extreme conditions, 30% salt concentration, pH 8.5, temperatures up to 50°C and anaerobic environment. It was discovered initially in the *Summer Lake* in Oregon by J.C. Raymond & W. R. Sistrom in 1967 [21], then later in 1978 in the *Wadi Natrun* in Egypt. Their purple color comes from bacteriochlorophyll, enabling them to live on photosynthesis. However in contrast to algae they cannot reduce water but rely on H₂S as a source of hydrogen. This anoxygenic photosynthesis does not lead to the production of O₂ but of elementary sulfur, which is excreted in the form of small spheres sticking to the cell wall (therefore the name *ectothio-*). It was a surprise to T. E. Meyer in 1985 when looking for colored proteins in these purple bacteria to discover a yellow protein [22]. In 1990 he also found PYP in two other halophilic purple bacteria, and in 1996 the gene of PYP was found in *Rhodobacter sphaeroides* [23].

PYP is believed to act as a photoreceptor responsible for the phototaxis of the bacterium *Ectothiorhodospira halophila*. It is attracted by red or infrared light and repelled from blue light. It swims by the use of a flagellum. In the absence of blue light it moves straight most of the time and changes direction only occasionally. The presence of blue light increases the frequency of direction changes. This simple mechanism leads to a net migration away from blue light. The wavelength dependence of the negative phototaxis action spectrum roughly matches the absorption spectrum of PYP [24].

PYP is a relatively small, water-soluble protein with a single chain of 125 residues and 14,000 Dalton molecular weight. Its fold is shown in figure 24. It has a chromophore, called *4-hydroxy-cinnamic acid* (also called *para-coumaric acid*, figure 25), covalently bound to the protein, which accounts for its yellow color. The chromophore is buried in the hydrophobic core of the protein with no atom exposed to the solvent. PYP is a

very stable protein. It is easy to crystallize and its crystals are very stable, well ordered and diffract to high resolution.

Upon absorption of a photon it undergoes with 35% probability a reversible photocycle with a total length of about one second (figure 26). Four photocycle intermediates, characterized by their different visible absorption spectra (figure 27) are known. Probably the most long-lived (I_2) state is the signaling state. However, it is not known yet, with which signal-transducing molecule in the cell it interacts. The primary event, which triggers the photocycle, is apparently an ultra-fast photo-isomerization about the double bond of the chromophore.

X-ray diffraction studies of a PYP photocycle intermediates have already at the National Synchrotron Light Source in 1993 [25]. A 10-ms pulsed Laue experiment led to a structure of the I_2 state showing a large-amplitude motion of the aromatic ring of the chromophore. The motivation for continuing the PYP Laue diffraction studies at the ESRF was to extend the time-resolution to the earlier intermediates.

7.5. Single bunch experiments on PYP

The PYP crystals were excited at a wavelength of 495 nm with laser pulses of 7 ns FWHM. These pulses were made by a Nd:YAG pumped dye laser. For this experiment the laser dye Coumarin 500 was pumped by the third harmonic of the Nd:YAG laser, 355 nm. The output of the dye laser, 11 mJ per pulse, was injected into an optical fiber of 1 mm diameter and guided to the diffractometer over 6 m distance. At the output of the fiber the light was refocused to the sample by a lens into a spot of about 1.5 mm in diameter, which contained a pulse energy of 4 mJ.

The crystals were mounted in sealed X-ray capillaries. During the experiment the sample was in a cooled air stream of 8 °C in the X-ray diffractometer.

To isolate single X-ray pulses, the prototype X-ray chopper by Wilfried Schildkamp was used. The timing between the exciting laser and probing X-ray pulse was verified by an avalanche diode (Hamamatsu S2384) placed at the sample position. Data sets at time delays 1 ns, 5 ns, 10 ns, 59 ns and 422 ns were collected in single bunch mode with 100 ps X-ray pulses; and at time delays of 1.5 μ s, 8 μ s, 50 μ s and 350 μ s in 1/3 filling mode with 0.9 μ s X-ray pulses. The data was collected with laser excitation alternating on and off, recording two images at the same orientation of the goniometer spindle. For each image 10 pulses were accumulated before the detector was read out. An X-ray diffraction pattern is shown in figure 28.

The crystals were grown by Benjamin Perman at the University of Chicago from material provided by Klaas Hellingwerf, University of Amsterdam. The crystals are hexagonal, have a unit cell of $a = b = 66.9$ Å, $c = 40.8$ Å, $\alpha = \beta = 90^\circ$ and $\gamma = 120^\circ$ and space group $P6_3$.

The data have been processed by Benjamin Perman at the University of Chicago [26], using the *LaueView* software by Zhong Ren [18]. The electron difference maps were calculated using the experimentally measured Laue structure amplitudes of the ground state as reference. The phases for the structure factors were taken from the ground state model, omitting the chromophore. The electron difference map (figure 29), contoured at 4σ , is empty for most of the protein and shows strong features only close to the chromophore. Of the chromophore, the aromatic head group does not show strong displacement. There is a negative hole (feature *M*) at the place of the carbonyl oxygen of the thioester bond and a positive peak (feature *L*) at the opposite side of the chain.

The experiment showed that with nanosecond laser excitation, non-destructive, reversible excitation to a degree of 15% could be achieved. Photo-isomerization of the chromophore could be seen completed after 1 ns, although there might be still open questions about the precise geometry of the chromophore. The

amplitude of the difference peaks in the electron density map is only of the order of 2 electrons, due to the low extent of excitation and the low atomic number of the atoms involved.

8. Future experiments with a Free Electron Laser(XFEL)

The use of single-bunch techniques on synchrotrons has made it possible to conduct a large number of pump and probe experiments in physics, chemistry and biology with time resolution down to 50 ps. Moreover, by the use of time resolved detectors such as a jitter free streak camera, it has been possible to study perturbations and phase transitions on the surface of semiconductors with 1-10 ps resolution. The most striking examples of the use of pulsed synchrotron radiation are the Laue studies on smaller macromolecules (MbCO [7], HbCO and PYP [26]). In these systems one can track changes in the three dimensional structure with 100 picosecond time resolution. The main difficulty has been that proteins are quasi opaque at optical wavelengths, which makes it difficult to excite the entire volume of the crystal. In practice, most systems have been partially excited to only 10-20 %. Note that a displaced carbon molecule ($Z=6$), populated to 15% will diffract less strongly than a hydrogen atom in the ground state. At low excitation levels, one is forced to accumulate many pulses of x-rays to improve the signal to noise. The signal-to-noise ratio of difference amplitude (excited minus non-excited state) is proportional to $N_{\text{ex}} \times N^{1/2}$, where N_{ex} is the number of excited molecules and N the number of incident x-rays.

Ideally, one would like to have one photon absorbed per active site. In practice 100% excitation may be hard to achieve with an ultra short pulse (which will be of interest for an XFEL). At the 100% flux level, i.e. the pulse energy is set to excite 100% of the active sites, multi-photon absorption will occur which may result in stimulate emission of sending the active site back to the ground state. Consequently, it may prove difficult to excite more than 50% of the sites using ultra-short pulses. However, in dealing with ultra short pulses, the pulse has a natural bandwidth. By suitable optics it may be possible to generate a chirp such that the longer wavelength arrive before shorter wavelengths. In this case one may exceed the 50% limit. To the extent that one can choose the excitation wavelength, one would choose a wavelength where the ground-state absorbance is modest (ca. 10 %) to minimise longitudinal gradients and a wavelength where the absorbance of the photoproduct is smaller than the ground state (transient bleaching). The front of the pulse can thus pave the way for back.

Recently small organic crystals undergoing ultra-fast, light triggered phase transitions were excited with fs laser pulses at a frequency of 900 Hz and large relative changes in the diffraction amplitudes were observed. It would be very interesting to see if this approach can be extended to proteins. For such proteins, the Laue techniques could be replaced by monochromatic oscillation diffractometry with a significant gain in spatial resolution. It seems important to study the efficiency of near-infrared excitation including the use of two-photon absorption.

From table 9 it is seen that an XFEL undulator gives 1×10^{13} ph/0.1%bw/pulse in a 200 fs pulse as compared to 3×10^8 ph/0.1%bw/pulse from a 200 ps synchrotron pulse. The XFEL delivers thus 3×10^4 more photons per pulse than the ESRF. Note that the line width $\delta E/E$ of the XFEL fundamental is 1×10^{-3} as compared with to 1-15% of an ESRF undulator. An XFEL is spectrally pure and close to the 1.4×10^{-4} bandwidth of a silicon(111) crystal. However, the XFEL peak is sitting on a very broad polychromatic background at 1×10^8 ph/0.1%bw/pulse, close to ESRF peak levels! One is likely therefore to maintain conventional monochromators on an XFEL.

The bunch structure of the XFEL at Desy consists of a 1.0 ms pulse containing 12000 mini pulses each separated by 80 ns. The next train arrives 200 ms later. In the following we will indicate how present synchrotron methods can be implemented on the XFEL at Desy.

Laue diffraction: An XFEL is not meant for Laue diffraction due to its monochromaticity. However, the SASE amplification can be quenched by a taper in the undulator gap and the background, which will correspond to a classical tapered undulator, can be used for Laue diffraction (high energies should be removed by a mirror). The unique feature of Laue diffraction is that the intensity is fully recorded by the polychromatic beam. A much better alternative is to induce a vertical dispersion of the monochromatic beam using a beryllium lens. Matching the vertical divergence to the crystal mosaicity will give the full intensity from a stationary crystal from the fully coherent beam. The signal to noise ratio and resolution should improve substantially. The 5 Hz repetition rate is adequate for this technique since the protein crystal needs to cool down between laser shots. In general it would be to have an accumulating 2D-detector which could pick-up say the first 100 frames in the pulse train. As an example, the first frame could be a 200 fs time point, the second a 80 ns, the third a 160 ns etc. That would save time and the radiation damage from the laser.

Small molecule diffraction: The experience from ESRF has shown that some electron transfer crystals that can run at frequencies up to 1000 Hz. That makes optimal use of our chopper and femtosecond laser and makes conventional monochromatic data collection with a 2D detector possible. We are expecting that this technique will give us 100 ps stills of the electron density at atomic resolution (1.2 Å). The 5 Hz repetition of the XFEL is non optimal. Nevertheless the global data collection time will be shorter by a factor of ca 150 as compared to the ESRF.

Gas Phase Reaction [27]: The fastest reactions are found in gas molecules free of friction from the environment. The association of molecules is usually studied using cold molecular beams where the reactants are brought together by weak Van de Waals forces. Intermediate states and the reaction product will show up on an area detectors as rings of diffuse scattering. The radial distribution function holds contains information about the chemical composition, bond lengths and their time dependence. In dissociation reactions all bonds are stretched synchronously and interesting effects such as the transition from ionic and covalent bonding may be observed. In general the interpretation of these experiments needs input from a model and theory. Gas phase reactions take place at low densities (pressures) and one would likely have liked to be accumulated at higher repetition frequencies, e.g. 1000 Hz!

Liquids: The formation of a cage around photo-active molecules determines the rate constants of a reaction. The cage may trap and moderate the reaction products and hence promote recombination, which sometimes passes through hot molecular states. It would be of great value to measure the pair correlation function and determine the geometry and composition of the cage. In irreversible reactions, the liquid is delivered through a laminar jet at a speed such the sample is exchanged between shots. Here again 5 Hz accumulation is non optimal.

Surface melting: When the surface of a semiconductor is hit by an ultra short laser pulse, its reflectivity changes from that of a solid to a liquid. That has been interpreted as evidence for ultra fast surface melting. By combining surface and bulk diffraction, it should be possible to check the structural transition following non thermal melting induced by valence band electrons. The scientific case for surface melting is discussed in these proceedings by J. Larsson et al. The diffraction process may be recorded by a gated detector

(avalanche diodes with a boxcar integrator). In this case one may use two x-ray pulses 80 ns apart. The first measures the non-excited state and the second the excited state. Collecting the two states quasi simultaneously should reduce the $1/\omega$ shot-noise.

X-ray spectroscopy: On the shortest times scales, the structural rearrangements are fairly localised and a local probes such as EXAFS is a powerful probe especially in disordered systems of low complexity. The energy scan may be accomplished by a scan of the undulator gap or by changing the electron energy of the bunches, which is a fascinating new feature of an XFEL. The normalisation of these experiments is sensitive to the stability of the x-rays, the laser, the sample and the detector.

The time resolution in future pump and probe experiments on an XFEL undulator, is determined by the convolution of the x-ray and laser pulse length and their relative jitter. Note that the timing jitter between the laser and the x-rays at the ESRF is 3-5 ps (RMS). A femtosecond laser on an XFEL beamline will have to be seeded by the same laser that is used to create the electron bunches. And these pulses will have to be transported in vacuum to the beamline (in the x-ray pipe?).

The time resolved x-ray community is small but growing steadily. In order to strengthen the scientific case for a free electron laser, we encourage people to harvest the potential of existing picosecond facilities such as the ESRF, APS and SPring8.

Acknowledgements

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Figure captions:

figure 1: Bunch modes at the ESRF. The availability, maximum current and lifetime is also shown. The lifetime is defined by the exponential decay $I = I_0 \cdot e^{-t/\tau}$.

figure 2: The x-ray pulse length at ESRF measured by an optical Hamamatsu streak camera. With permission from Kees Scheidt, ESRF.

figure 3: The spectral flux of the undulator U17 from *one* pulse from the 16 mA single-bunch mode. The undulator is at 6.0 mm gap. The flux is calculated for a 6.0 x 1.4 mm aperture 30 m from the source. The integrated flux is 3.25×10^{10} ph per pulse.

figure 4: The spectral flux of an undulator is proportional to $Q_n(K)$. For K greater than 5, the device becomes a wiggler with a quasi-continuous spectrum.

figure 5: The central brightness of an undulator is proportional to $F_n(K)$. The curves give the amplitude distribution of the first, third, fifth, seventh and ninth harmonic.

figure 6: The in-vacuum undulator period and K vs the undulator fundamental.

figure 7: Comparison between undulators designed for 6 and 16 mm gap respectively. The undulators are 2.0 m long. The single-bunch current is 16 mA.

figure 8: The spectral flux of the focused of the insertion devices on ID09 (March 2000).

figure 9: The beamline optics on ID09. Note the fast shutter in front of the toroidal mirror (March 2000).

figure 10: Cross section through the vacuum vessel of the Julich chopper.

figure 11: The schematic layout of position monitors, shutters, slits and diffractometer.

figure 12: The wire monitor, ms-shutter and chopper. These components are installed in the beamline vacuum.

figure 13: The procedure for the selecting a single x-ray and laser pulse in a Laue experiment.

figure 14: The synchronisation of the chopper and femtosecond laser to the RF clock.

figure 15: The single-circle diffractometer for pump and probe experiments. The laser excitation is here configured in an orthogonal geometry. Note the motorised laser telescope, which is used to scan the laser position into the interaction point, i.e. the crossing between the x-ray beam, the laser beam and the diffractometer axis (the crystal).

figure 16: The myoglobin molecule and its binding site. The drawing on the left shows a ribbon representation of the protein backbone with the CO molecule on top of the heme plane. A realistic space filling is shown on the right where each atom is shown with its Van-der-Waals radius. The molecule is rendered with the program Molscript and the data taken from Kurian, Karplus & Petsko 1986-87, PDB 1mbc.

figure 17: Sample environment in the femtosecond spectrometer. The pump beam illuminates the entire sample, a crystal of 100-200 μm size, whereas the probe is tightly focused.

figure 18: Evolution of the MbCO spectrum after flash photolysis. Transient absorption spectra of a 300- μm size MbCO crystal (P21). Shown is the difference in absorbance relative to the spectrum of the unexcited sample. The top trace is shown as a reference only.

figure 19: Difference between the MbCO and deoxy spectra in solution. The difference spectrum is similar to the signature observed in crystals 10 ps after excitation. (Solution data provided by Philip Anfinrud, Harvard University, 1999)

figure 20: Single Pulse Laue experiment on myoglobin. The exciting laser pulse arrives 500 ps in advance of the probing X-ray pulse. Circularly polarised laser excitation collinear to the rotation axis guarantees that the excitation degree is the same for all angular settings.

figure 21: Laue diffraction pattern of a myoglobin crystal. 64 single X-ray pulses with 500-ps time delay to the laser pulse are accumulated by detector at a repetition rate of 0.5 Hz. Detector: liquid-nitrogen cooled CCD with X-ray image intensifier, 125 mm distance, X-ray source: U46 and W70.

figure 22: The photolysis and rebinding of the CO ligand in myoglobin. This series of difference maps is based on pulsed Laue data collected at the ESRF on the ID9 beamline [Srajer et al. 1996]. In the 4 ns map, the positive blue features around the red hole left by the CO, could be indications of docking sites. Electron density maps are contoured at $+3.5\sigma$ (blue) and -3.5σ (blue). Picture prepared by Thomas Ursby, ESRF.

figure 23: Zoom of the 4 ns difference map from figure 22.

figure 24: Structure of Photoactive Yellow Protein. Shown is the Protein backbone and the chromophore 4-hydroxy-cinnamic acid attached to Cys-69 and the protein envelope. (PDB entry 2PHY [28], Molscript 2.1.2 © Per Kraulis, 1998)

figure 25: The chromophore of PYP, 4-hydroxy-cinnamic acid. It is covalently bound to the cysteine-69 residue by a thioester bond.

figure 26: Photocycle of the photoactive yellow protein. In the ground state P the chromophore is in the trans conformation; but in the intermediate states starting with I_0 , apparently in the cis conformation. P stands for PYP, I for intermediate. The wavelength is the center of the absorption band. Alternative nomenclature: P = pG (PYP ground state), I_1 = pR (PYP red shifted), I_2 = pB (PYP blue shifted).

figure 27: Absorption spectra of PYP photocycle intermediates. The spectra of the P ground state, I_1 and I_2 are taken from Hoff [29], the spectra of I_0 and $I_0^\#$ from Ujj [30].

figure 28: Laue diffraction pattern of PYP. The sample is a $100 \times 100 \times 200 \mu\text{m}^3$ crystal by Benjamin Perman, University of Chicago. For this image, 10 single pulses of 100 ps have been accumulated over 46 s. The X-ray pulse arrived 1 ns after the peak of the stimulating laser pulse of 7 ns. This image contains about 3700 useful reflections. (image pypb35_017, 27 Nov 1996 14:58, source: W70, 20 mm gap, detector: XR11 / CCD, distance: 150 mm).

figure 29: 1-ns difference map of PYP. In blue regions the electrons density electron density increases relative to the ground state, in red regions it decreases. Contoured at 2.6σ . Right: Modeling of excited state, by Benjamin Perman, PDB 2PYR [26]. (Graphics by XtalView 3.2 © Duncan McRee)

Tables:

table 1. Source parameters of three third generation synchrotrons. The bunch length us quoted in RMS units($fwhm=2.355 \sigma$).

Source	ESRF	APS	SPring8
Energy (GeV)	6	7	8
Circumference (m)	844.1	1104.0	1436.0
Revolution time (\square sec)	2.816	3.683	4.790
Harmonic number(RF)	992	1296	2436
Radio frequency(MHz)	352.2	351.9	508.6
Min-bunch spacing(ns)	2.839	2.842	1.966
Multi-bunch current(mA)	205	100	100
Multi-bunch lifetime(hours)	55	40	110
Single-bunch current(mA)	16	5	15
Single-bunch lifetime(hours)	6.5 (8 mA), 6 (15 mA)	8.1. ?	11(1mA), 2(15mA)
Zero current bunch length σ (ps)	25.5	44.6	17.0
No of insertion device beamlines	29	35	38
Length of straight section(m)	6.1	6.7	6.65(30)
Undulator length(m)	3 * 1.6	2* 2.4	1* 4.5(30)
Minimum undulator gap(mm)	6.0	10.5	8.0

table 2. Electron source parameters of third generation synchrotrons. Distributions are quoted as rms.

Source	ESRF(low- β)	ESRF(high- β)	APS	SPring8(high- β_x , low- β_z)
ϵ_x (nm rad)	3.8	3.8	8.0	6.0
ϵ_z (nm rad)	0.011	0.011	0.08	0.006, 0.0024(bare lattice)
coupling (ϵ_z / ϵ_x)	0.003	0.003	0.01	0.001, 0.0004(bare lattice)
σ_x (μ m)	56.5	391.7	325.0	366.4
σ_z (μ m)	10.2	9.8	86.0	4.7
σ_x' (μ rad)	87.6	10.4	23.0	14.9
σ_z' (μ rad)	3.8	3.9	9.0	1.17

table 3. Single pulse performance of in-vacuum undulators at the ESRF. The undulator is 2.0 m long, based on SmCo magnets and operates at 6 mm gap. The spectral flux is calculated for a single-bunch current of 16 mA.

period(mm)	poles	E _r (keV)	K	P(kW/200 mA)	I ₁ (ph/0.1%bw/pulse)	I ₃ (ph/0.1%bw/pulse)
20.1	200	10.0	1.21	3.84	4.24E+08	1.04E+08
16.9	236	15.0	0.86	2.71	3.53E+08	3.84E+07
14.6	275	20.0	0.62	1.89	2.60E+08	1.06E+07
11.0	363	30.0	0.31	0.82	1.03E+08	3.53E+05

table 4. The present insertion devices installed on ID09, March 2000. The size of the electron beam the low- β site is 0.117 mmh and 0.024 mmv (fwhm). The U17 in-vacuum undulator will replace the U20 and W70 in July 2001.

ID	poles	minimum gap (mm)	E _r (keV)	E _c (keV)	K	P(W/200 mA)
U20	162	16.3	16.70	3.5	0.27	160
U46	71	16.0	0.64	15.6	2.76	3116
W70	43	20.1	0.32	20.0	5.43	4788
U17	235	6.0	14.84	13.2	0.86	2740

table 5. The monochromatic beam from the Si(111) monochromator in single bunch mode(16 mA). The energy is 16.45 keV, the fundamental of the U20 undulator

Tunability range(keV)	4.9-39.6
Resolution(eV)	4.3
Focal size(mmh x mmv)	0.18 x 0.20
Flux(ph/s @ 200 mA)	1.8×10^{13}
Flux(ph/pulse)	4.1×10^6
Stroboscopic flux(ph/s @ 896.6 Hz)	3.6×10^9

table 6. The mirror parameters

-	mirror 1	mirror 2
shape	cylindrical(toroidal)	plane(parabolic)
dimensions : L x W x H(mm ³)	1000 x 130 x 100	1200 x 100 x 50
material	SiC on a graphite body	Si(single-crystal)
sagittal radius(mm)	64.53	-
coating	Pt	Pt
surface roughness(Å; rms)	1.8	1.0
incidence angles (mrad)	2.335	1.25-5.0
energy range(keV)	5-38	5-55
source-mirror distance(m)	28.1; 29.7; 31.3	33.9; 35.5; 37.1

mirror-focus distance(m)	26.8	7.0
demagnification M	0.85-0.95	0.2
slope-error(μ rad; rms)	9.0(4.1 over 380 mm in the centre)	6.0
gravity sag(km)	4.7	6.9
bending range(km)	1.8-4.7	1.5-6.9
pushing range(km)	4.7- ∞	6.9- ∞

table 7. The chopper parameters

Tunnel length(mm)	165.0
Maximum radius(mm)	96.8
Tunnel off-set(mm)	47.35
Minimum rotation frequency(Hz)	10.0
Maximum rotation frequency(Hz)	896.6
Tunnel width(mm)	4.0
Tunnel height(mm)	0.05 to 0.90
Minimum opening time: δt_{\min} (sec)	0.10×10^{-6}
Maximum opening time: δt_{\max} (sec)	0.17×10^{-3}
Phase jitter(sec); RMS	10×10^{-9}
Axial resonance frequency(Hz)	998
Centrifugal breakdown frequency(Hz)	1300

table 8. The performance of the Ti: Sapphire laser. The pulse length is 100 fs.

	wavelength(nm)	energy(eV)	energy(μ J/pulse)	ph/pulse	ph/sec
1 harm.	800	1.55	750	$3.0E+15$	$2.7E+18$
2 harm.	400	3.10	150	$3.0E+14$	$2.7E+17$
3 harm.	267	4.64	25	$3.4E+13$	$3.0E+16$
OPG/OPA	420	2.95	35	$7.4E+13$	$6.6E+16$
OPG/OPA	760	1.63	35	$1.3E+14$	$1.2E+17$

table 9. Comparison between the U17 undulator (low- β) and the expected performance of a free electron laser (XFEL) based on a 250 GeV linac at Desy.

-	ESRF	XFEL
fwhm bunch length(s)	150×10^{-12}	200×10^{-15}
Spectral flux(ph/pulse/ 0.1%bw)	3×10^8	1×10^{13}
Peak flux (ph/sec/0.1%bw)	2×10^{18}	1×10^{26}
Average flux (ph/sec/0.1%bw)	1.5×10^{15}	2×10^{18}
Undulator band width(%)	1-15%	0.1%

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