Plastique: A synchrotron radiation beamline for time resolved fluorescence in the frequency domain

Gelsomina De Stasio, N. Zema, and F. Antonangeli
Istituto di Stuttura della Materia del CNR, via E. Fermi 38, 00044 Frascati (Roma), Italy

A. Savoia
Sincrotrone Trieste, Padriciano 99, 34012 Trieste, Italy

T. Parasassi
Istituto di Medicina Sperimentale del CNR, viale Marx 15, 00134 Roma, Italy

N. Rosato
II Universita' di Roma "Tor Vergata," Dipartimento di Biochimica e Medicina Sperimentale, Via Orazio Raimondo, 00100 Roma, Italy

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PLASTIQUE is the only synchrotron radiation beamline in the world that performs time resolved fluorescence experiments in frequency domain. These experiments are extremely valuable sources of information on the structure and dynamics of molecules. We describe the beamline and some initial data.

Time-resolved fluorescence is one of the leading techniques in molecular dynamics.[1] Only in recent years, however, this technique has exploited the unique characteristics of synchrotron radiation. We describe PLASTIQUE, the only operating synchrotron radiation beamline for frequency-domain fluorometry. In particular, PLASTIQUE is the only fluorescence facility of its kind for near UV photons, with a total range of 200–800 nm (see Fig. 1).

A pulsed light source as provided by synchrotron radiation is uniquely suited for excitation in time-resolved fluorescence, because of the possibility of continuously varying the wavelength range, and because of the short duration and high repetition rate of the pulse.

The time structure of synchrotron radiation simultaneously provides a large number of modulation frequencies. PLASTIQUE is connected to the storage ring ADONE at the Frascati National Laboratory, whose single-bunch Gaussian light pulse contains a set of harmonic frequencies 2.8585 MHz apart ("comb function"), with a Gaussian-envelope half width of 500 MHz.[2] A pioneering work by Spencer and Weber made it possible to extract a single harmonic frequency with a powerful technique: the cross-correlation method.[3]

PLASTIQUE collects synchrotron radiation emitted by a bending magnet on the 1.5 GeV storage ring ADONE, over 2.5 mrad on the horizontal plane, and 4 mrad on the vertical plane. Figure 2 shows an overall view of the beamline.

On the sample the spot size is approximately 1 mm, in very good agreement with the ray tracing simulations performed during the project phase of the beamline.

We do not need ultra high vacuum, because light in our spectral range does not interfere significantly with air, and a low vacuum is sufficient to maintain the mirror surfaces clean. The high-energy cutoff is determined by the insertion of silica windows (7.75 eV). The low vacuum section is completely built using PVC pipes, inspirating the name of the beamline.[4] PVC (poly-vinylchloride) was chosen because it satisfies all mechanical requirements, and it is capable of maintaining a 10^{-3} mbar pressure.

A spherical grating monochromator (Jobin-Yvon H10 equipped with 1200 lines/mm grating, focal length 10 cm, $f = 3.5$) sends the beam to an optical module (SLM OP450) for multifrequency phase and modulation fluorometry (Fig. 3).[2,5]

A slave frequency synthesizer (Marconi Instruments 10 kHz–1 GHz signal generator 2022A) is in phase with the synthesizer that drives the rf cavity of the storage ring, thus with the light pulse repetition frequency. Note that the *slave* frequency synthesizer takes, as a reference signal, the output of the synthesizer that drives the rf cavity. This output is extremely stable and reproducible, about one order of magnitude better than the synthesizers usually used on conventional phase fluorometers, or in the first synchrotron radiation fluorometer built in Frascati in 1983.[2] It results in ten times more accurate phase measurements, or in ten times shorter acquisition time.

The output of the slave synthesizer can be varied in order to obtain a frequency equal to ADONE's fundamen-

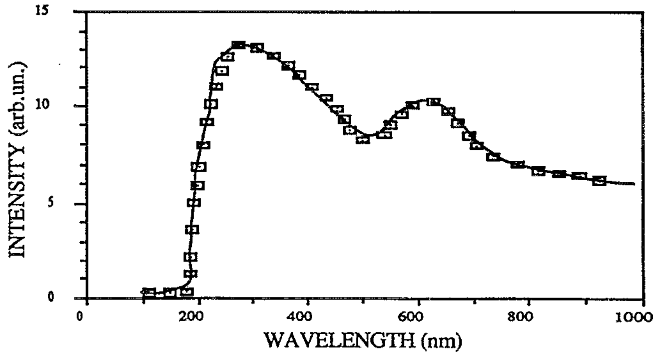

FIG. 1. The intensity spectrum of PLASTIQUE. The low-wavelength limit is determined by the cutoff of the fused silica window.

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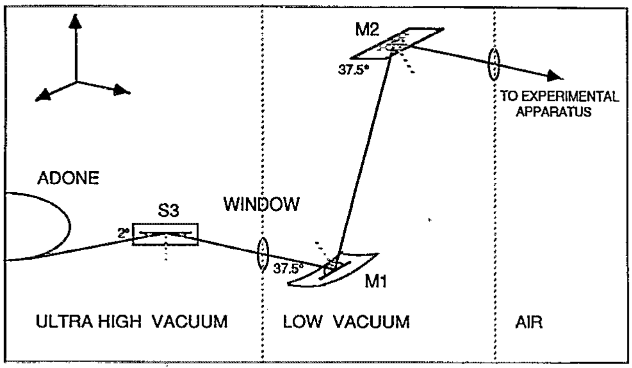

FIG. 2. The layout of PLASTIQUE on the Adone storage ring. Note the three sections: ultra-high vacuum, low vacuum, and air pressure.

tal, or to one of the harmonic components, plus 40 Hz. The small difference, 40 Hz, is the cross-correlation frequency. We can perform experiments up to about 330 MHz.

The outputs of the two PMTs are analyzed separately by two identical channels in the electronic analysis unit (ISS GREG 80) whose output is interfaced with an IBM PS 30 computer, that for each frequency calculates the demodulation ratio M

$$M=\frac{\mathrm{ac}_{\mathrm{em}}/\mathrm{dc}_{\mathrm{em}}}{\mathrm{ac}_{\mathrm{ex}}/\mathrm{dc}_{\mathrm{ex}}}. \tag{1}$$

The phase difference between the reference and the sample signal ϕ is measured by a digital phasemeter. The resolution for phase measurements is 1 μs, which corresponds to an angular resolution of about 0.01°.

From the two simple equations

$$\phi=\tan \omega\tau_\phi \tag{2}$$

and

$$M=(1+\omega^2\tau_M^2)^{-1/2}, \tag{3}$$

we can calculate the phase and modulation lifetimes τ_ϕ and τ_M.

PLASTIQUE has already been used for a variety of tests and experiments demonstrating that samples with 0.1 absorbance are clearly measurable. We present here a specific experiment.

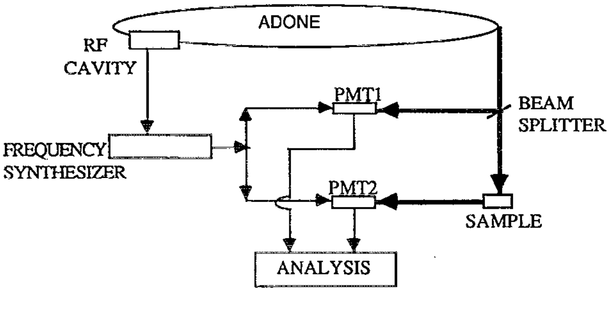

FIG. 3. A block diagram of the multifrequency phase and modulation fluorometer.

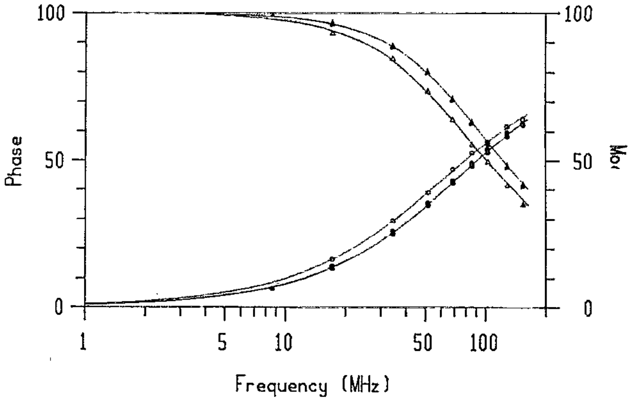

FIG. 4. Phase (circles) and modulation (triangles) values as a function of light modulation frequency, for holo (full symbols) and apo (open symbols) human copper-zinc superoxide dismutase. The solid lines correspond to the best fits.

Figure 4 shows phase and modulation data for a solution of a dimeric protein (human copper-zinc superoxide dismutase) containing two equivalent tryptophans.[6] Excitation was at several wavelengths ranging from 260–290 nm, with a bandwidth of 8 nm.

The data were analyzed with a model based on a continuous lifetime distribution demonstrating that the fluorescence decay cannot be described with a single lifetime, although the two tryptophans contained in the dimer are fully equivalent and both exposed to the solvent. A Lorentzian continuous lifetime distribution (centered at 2.2 ns and with full width at half maximum of 0.3 ns) is needed to obtain a good fit of the data.[6]

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[4] The word PLASTIQUE means indeed "plastic" in French. However, the name of the beamline was also interpreted as an acronym in Italian, for "Picclola Linea Accroccata Senza Tanta Immaginazione, Qualitativamente Un Errore," meaning "small beamline put together without much imagination, a qualitative mistake."

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