Reflection anisotropy spectroscopy of biological molecules with the 4GLS source

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1 Introduction This article gives an outline of the main features of a number of related experiments on biological systems that could be carried out on the UK Fourth Generation Light Source (4GLS). 4GLS (Fig. 1) is a suite of accelerator-based light sources, based on a 550 MeV Energy Recovery Linear Accelerator (ERL), which contains a VUV Free Electron Laser (FEL) as well as conventional insertion devices in the electron transport path. There is also an XUV FEL and an IR FEL, fully integrated with the sources on the ERL. 4GLS is optimised to deliver high brightness radiation in the range 3 to 100 eV, but will also provide very powerful sources of coherent radiation in the terahertz (THz) regime. The light output of 4GLS is summarised in Fig. 2 and a full description of the facility can be found on the 4GLS web site [1].

The 4GLS facility has remarkable potential to advance research programmes over a very wide range of science and technology. In order to realize this potential it will be necessary to design instruments to exploit the characteristics of the various 4GLS light sources. Since the light source capabilities of 4GLS are unprecedented this will necessitate the design of state of the art instrumentation carefully matched to the scientific problems to be investigated. This article explores a number of pump probe experiments on biological systems that will be possible with 4GLS.

The 4GLS research programme includes a number of Flagship Proposals that have been developed by leading

members of the UK scientific community and their UK and international collaborators. Four of these Flagship Proposals are concerned with research on biological systems. They are: “Protein structure and dynamics” lead by Professor David Klug (Imperial College), “Cell and tissue imaging” lead by Professor Paul O’Shea (University of Nottingham), “Biocatalysis, photosynthesis and membrane proteins” lead by Professor Nigel Scrutton (University of Manchester) and “Molecular assemblies in the extra cellular matrix and cell signalling” which is lead by Professor David Fernig (University of Liverpool).

2 Potential of 4GLS for studying mechanisms of biological organisation

A general feature of all these research programmes is an interest in the mechanisms by which biological molecules organise, self-assemble and carryout their functions. The scale and complexity of such self-organisation is illustrated by the folding of DNA [2, 3] (Fig. 3). The human genome contains some three billion base pairs in the form of a double helix that is two meters long. In the nucleus of a cell the DNA, which is divided into a number of chromosomes, is compressed into a two micron structure by an intricate system of folding processes [2, 3]. In order for the information contained in DNA to be read this compact structure must be unwound and subsequently rewound. From a thermodynamic perspective one expects these activities to be driven by the free energy released from chemical reactions but we

Figure 1 Schematic layout of 4GLS.

have very little understanding of the physical mechanisms involved. It is reasonable to suppose that these mechanisms will involve the vibrational and rotation modes that can be excited by thermal processes at the temperature at which this activity occurs which is just above room temperature. These modes will be in the THz region of the electromagnetic spectrum since at room temperature $kT \sim 6$ THz. A key issue is whether there are any long-lived coherent modes in such biological systems in this frequency range. Frohlich suggested many years ago on theoretical grounds [4–6] that such modes do exist and that they play an important role in biological organisation. However this view is controversial and there are good theoretical reasons to expect that such modes would rapidly dissipate their energy into the “thermal sea” of normal modes of vibration of such systems [7]. One might expect such thermalisation to occur on a picosecond timescale. The key to research in

this field is to conduct experiments but this has proved to be very difficult due to the absence of strong sources of radiation in this frequency range, the so called THz gap. Fortunately 4GLS is an ideal source to fill the THz gap since it is based on an ERL in which the electron bunches, which circulate only once, are quite short. When the bunch length is shorter than the wavelength of the emitted light, a condition satisfied at ~ 1 THz, this gives rise to a dramatic increase in intensity due to the phenomena of coherent emission [8]. A laboratory source for example would yield an average power of $\sim 100 \mu\text{W}$ in the THz region of the spectrum whereas 4GLS will provide average powers of ~ 2.6 kW and peak powers of ~ 100 MW. With such high powers available it will be possible to search for long-lived modes that might be involved in the self-organisation of biological molecules. There have been a few recent indications that such long-lived modes do exist in biological systems [9, 10].

Figure 2 Average flux per 0.1% bandwidth for different sources on 4GLS. Note the undulator and wiggler curves are example devices only.

However in searching for such modes it is unlikely that simple spectroscopy will be useful since in the THz range biological molecules will have a large number of normal modes and many of these will be populated by the thermal energy distribution of the ground state. This is expected to give rise to a broad featureless THz spectrum as shown recently for hen egg white lysozyme and horse heart myoglobin [11]. Furthermore although the problem of multiple occupation of modes in the ground state will be reduced at shorter wavelengths infrared spectra are dominated by intense short range modes and the long range modes that should reveal information on biological organisation are very much weaker.

The key to research in this field is to search for the response of a biological molecule to a variety of excitations, including the direct excitation of long-range vibrational modes, and this is a role for which 4GLS, with its wide range of synchronised sources, is ideally suited. We now describe a number of proposals for “pump-probe” type experiments that are unique to the scientific case for 4GLS. The uniqueness arises from the plan to use the technique of Reflection Anisotropy Spectroscopy (RAS) [12] as a probe of the response of biological molecules to various excitations.

3 Reflection anisotropy spectroscopy RAS is an optical probe that achieves remarkable surface sensitivity through a subtle geometrical arrangement [12]. It solves the problem caused by the large penetration depth of light into solids which, in most optical techniques results in a reflected signal dominated by bulk contributions, by measuring the difference in reflectivity in two directions at right angles in the surface of normal incident plane polarised light. For a cubic substrate the bulk contribution then cancels by symmetry and the signal arises solely from the surface.

Recent work has demonstrated the potential of RAS to monitor conformational changes in biological molecules adsorbed at metal/liquid interfaces. It has been used to determine the three dimensional orientation of a small molecule adsorbed at a metal/liquid interface [13] and to distinguish between long sequences, ~ 3000 bases, of single and double stranded DNA adsorbed at metal/liquid interfaces [14]. It has been shown to have potential for the study of interactions between peptides and phospholipid membranes important in the pathology of toxic self-assembled amyloid fibrils implicated in Alzheimers disease [15]. It can also monitor interactions between molecules in real time [16] and has recently been used to monitor conformational changes in proteins induced by electron transfer [17].

However if the potential of RAS as a probe of biological molecules on 4GLS is to be realised a number of practical problems need to be overcome. Current laboratory instruments make use of a photoelastic modulator (PEM) that operates at 50 kHz and this imposes a fundamental limit on the speed of an RAS measurement of 20 μsec . In practice this limit is never reached due to the weakness of

wide band laboratory sources which cause the speed of the experiment to be limited by signal to noise considerations. An additional problem with laboratory instruments is that the output of most discharge lamps falls dramatically beyond 5 eV and this makes it impossible to reach important transitions to higher energy in DNA and other molecules.

Major advances in Circular Dichroism (CD) have followed from the extension of the spectral range of the technique into the UV by the use of bending magnets on synchrotrons. Similar advances arising from the extension of the spectral range of the technique would be possible in RAS by its incorporation on synchrotron sources. However significantly superior performance to that achieved on a synchrotron would be possible using a bending magnet or the Wiggler on 4GLS. This arises from the fast pulsed nature of 4GLS which has the potential to increase the signal-to-noise in these experiments by many orders of magnitude, provided the electronics are adapted to fully exploit 4GLS.

Figure 3 Steps by which DNA is packed into chromatin. (From H. Schiessel, J.Phys.: Condens. Matter **15**, R699 2003).

4 Reflection anisotropy spectroscopy on 4GLS

At first glance, the average brightness and the spectral range of the light available from a 4GLS bending magnet or the Wiggler might seem the limit of its value. However because this light is pulsed it allows gated electronics to be used, which can sample over a small interval while the light is present. This greatly improves the signal-to-noise ratio of the detector.

The aim of this approach is to capture complete spectra from single broadband pulses from a bending magnet or Wiggler. The pulse width of the light would be approximately 150 fs. The repetition rate could ultimately be as high as 1.3 GHz, although this approach would initially be developed at a lower repetition rate.

The high flux available from a wide band source such as a bending magnet or a wiggler on 4GLS will remove the signal to noise limitation on an RAS experiment and allow measurements on a nanosecond timescale provided a suitable apparatus, omitting the PEM, can be designed. Fig-

ure 4 shows a possible design. The beam from a bending magnet in the high average current loop of 4GLS is split with a half silvered mirror. One of the two resulting beams is sent on a longer path than the other before being recombined. It is noted that the polarisers have to be placed before the final half silvered mirror used for recombining the beams. This is not ideal as this mirror may modify the polarisation of the light. However it will be possible to calibrate for this effect. In this method the two polarisation components are not analysed at exactly the same time but maybe a nanosecond or so apart, depending on the detector limitations. The beams are reflected off the specimen at near normal incidence and near normal reflection and separately detected using fast gated electronics. Assuming the detector could discriminate pulses less than a nanosecond apart, this would allow sampling up to the full 4GLS repetition rate of 1.3 GHz. This would be similar to operating a conventional instrument with a PEM running at 1.3 GHz instead of 50 kHz, that is 260,000 times faster. In addition the extra light delivered in 150 fs pulses would dramatically increase the signal/noise. This method has the additional advantage over instruments using a PEM that the polarisation modulation is independent of the wavelength.

Figure 4 Design of RAS instrument on a bending magnet of 4GLS.

The higher flux available on 4GLS, the fast timing and the capability of performing a variety of pump probe experiments make it possible to envisage studies of kinetic effects on much faster timescales than is possible with existing laboratory equipment. Recent experience of applying RAS to studies of DNA, DNA bases, amino acids and proteins adsorbed or bonded to metal/liquid interfaces [13, 14, 16, 17] suggests a number of areas in which major advances would follow from such studies.

5 Biological systems: THz and water An important consideration in the study of biological molecules is that they need a liquid environment, usually water, in order to exhibit their functional behaviour and this may pose a problem if such studies involve THz radiation due to the very high absorption of THz radiation by the hydrogen bonding network of water: 1 mm of water attenuates radiation of 1 THz by a factor of 10^{18} . However the high THz absorbance of bulk water falls off for thin layers (~ 10 nm) and varies with ionic concentration making it possible to use THz radiation to make non-invasive measurements of ionic concentration in living tissue [18]. Furthermore the influence on biological systems of bound water and water in the surface layer of biological molecules are worthy of study in their own right since they have important effects on the dynamics of biological molecules [19]; water dipoles might be expected to couple to the vibrational modes of membranes and to the modes excited in the non-radiative decay of UV excited base pairs in DNA for example [20, 21]. Such studies are difficult but they are not impossible as shown by recent work on the THz absorption of proteins in water [22] and on THz near field imaging of live neurons in water rich specimens of ~ 100 μm thick in which the key issue was to detect the transport of water and Na ions between the intraaxonal and extracellular environments [18]. It has been possible with laboratory sources to study biological molecules in films of water 150 μm thick [19] and such studies will be much easier with the high power levels available on 4GLS. However it will be important in the design of such experiments to control the timing of the experiment so as to exploit the high peak power available in the THz range with 4GLS in order to distinguish the results from heating effects resulting from the high average power. Given the high power levels of the 4GLS THz sources it might be possible to saturate the absorption process with an initial pulse so that the material was transparent to the second pulse. This would depend on the lifetime of the initial excited state. The pulse structure, polarisation and coherence of the THz might also be exploited in the development of THz detectors.

6 Studies of damage mechanisms in DNA DNA damage from irradiation with UV light induces genotoxic, mutagenic and recombination lesions in DNA including single strand breaks, base damage and multiple damage sites. It is known that these lesions are induced by secondary species generated by the primary radiation and that shape resonances arising from the attachment of low energy electrons play a crucial role. Single strand breaks and the detachment of anions in particular show a pronounced dependence on the energy of low energy electrons, < 4 eV [23, 24]. The instrumental developments we envisage will make it possible to monitor the evolution of radiation damage in DNA as a function UV irradiation. Damage events would be initiated by irradiation using the VUV or XUV FELs, tuned to precise wavelengths to vary electron attachment energies, and the timescale of changes in DNA

conformation monitored using RAS. These experiments could also include the induction of conformational changes by controlled excitation of long range modes of vibration using THz radiation. The relationship between DNA conformational and the initiation of damage has recently been demonstrated [25] in experiments that have shown that the most common form of UV damage, the dimerisation of adjacent thymine bases, is complete within 1 picosecond of UV absorption. Thus in this case UV damage occurs too quickly to involve significant conformation rearrangement and depends purely on the local conformation at the instant of UV adsorption.

The individual bases and also the Crick and Watson base pairs in DNA are less susceptible to radiation damage than similar molecules and the non-radiative de-excitation processes that occur in DNA are known to involve conformational changes that should couple to the local environment through vibrational modes [20, 21]. The experiments that we envisage will make it possible to explore this coupling and may provide insight into the evolutionary processes that resulted in the “choice” of bases employed in DNA.

7 Studies of the local dynamics of biological molecules It is known that the excitation of probe molecules that undergo a large change in dipole moment in the excited state exert a force on the local environment and this has been exploited in the time resolved Stokes shift technique to measure the local relaxation dynamics in DNA sequences [26]. A combination of this technique, using initial excitations from the VUV or XUV FEL's, with the sensitivity of RAS to the orientation of DNA bases should yield useful insight into the dynamics of DNA molecules. It may even be possible to avoid the need for the probe molecule by careful choice of sequences and arranging to excite a particular dipole transition in a specific base in a well defined local environment. However we do not yet have the understanding of the relationship of the directions of dipole transitions to the molecular axes of DNA bases and to local environments that is necessary to design such an experiment though this is an active area of theoretical and experimental work [13, 14, 27–29].

It has already been demonstrated the RAS can be used to monitor conformational changes in proteins resulting from electron transfer processes [17] and in unpublished work we have detected changes in the RAS response of lipid layers caused by the interaction with peptides and proteins [15]. These observations indicate that it might be possible to obtain a fuller understanding of the mechanisms of biological function through the direct excitation of vibrational modes in THz pump-RAS probe experiments.

8 Conclusion We have described the potential of RAS to monitor the effects on biological molecules of excitations with a number of the light sources available on 4GLS. This approach is of immediate relevance to studies of DNA damage and DNA, protein and membrane dynam-

ics. It will also be of benefit in other areas of the 4GLS research programme such as real time studies of semiconductor growth, molecular interactions in the high pressure regime characteristic of real catalytic processes and the monitoring of fast stress related changes in metal systems important in aerospace [12].

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