Biology

Gold nanoparticle uptake and mechanism elucidation

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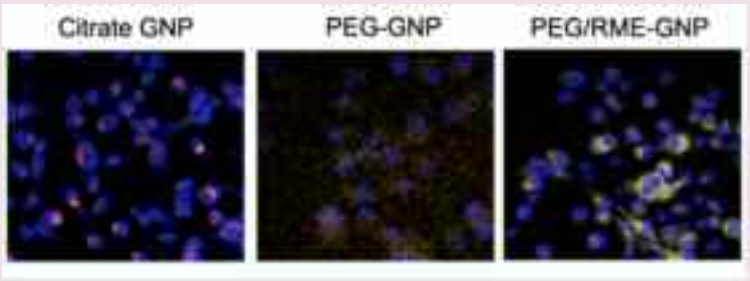
A technique has been developed to image the nanoscale distribution of gold nanoparticles within cells using short intense laser pulses. Unlike previous techniques, it is sensitive to the gold itself rather than some other chemical group attached for the purpose of imaging making the technique very general in nature. Furthermore the images created can be readily overlaid with images showing other aspects of cell structure or function. The technique has been used to determine the uptake dynamics of gold nanoparticle with various coatings across a range of cell lines and to infer the affect these gold nanoparticles can have on the mitochondria (the cell's energy factories). This research is informing the development of a new form of cancer therapy whereby the gold, preferentially taken up by tumour cells, will enhance the effect of radiation.



MPR and confocal images of MDA-MB-231 cells were treated with GNPs with various coatings (indicated) for 6 hours prior to fixing. DAPI shows the nuclei (blue) and GNPs density is shown in a heat-map (low density: green to high density orange).

The use of FLIM to measure the stabilities of metal complexes in living cells

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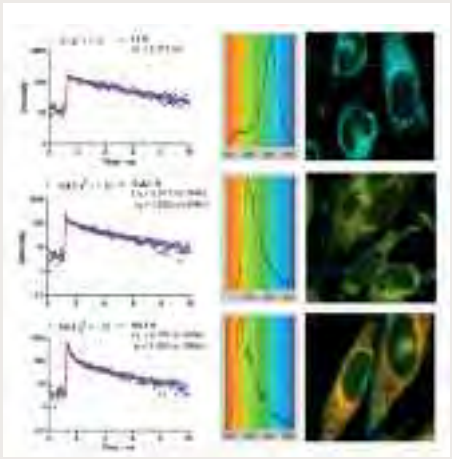
Metal complexes are widely used in medicine for both imaging and therapy. Many of these depend on intracellular processes to achieve selectivity but since the majority of analytical methods focus on the metal, little is known about the speciation of such species in living cells. We have designed a series of fluorescent metal complexes which can be followed in cells by virtue of their 1 or 2-photon fluorescence. While the the emission wavelength of a ligand may not change when a metal is coordinated the emission lifetime does and this provides a potential method to determine when and where dissociation of a metal complex occurs within a cell. We have explored the use of this approach to determine if and when intracellular demetallation of biologically active metal complexes occurs.



Sample point decay curves and lifetime cell maps for ligand L1 and its complexes with copper and nickel. The colours in the cell map are representative of the lifetime distributions at each point in the cell.

The plant secretoryome Part II: Golgi stack N-glycosylation enzymes interaction studies using fluorescence lifetime imaging microscopy

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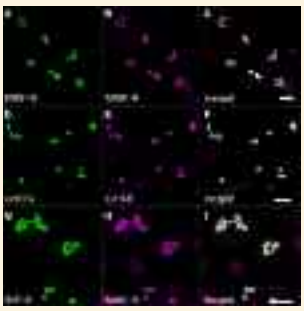
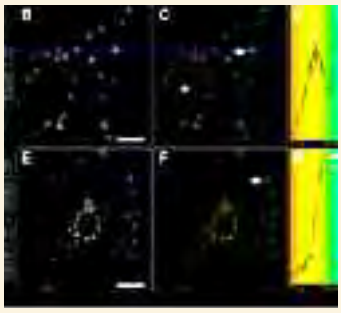
The plant cell Golgi apparatus is a key organelle that modifies, sorts and packages proteins. Its structure and mobility are thought to determine its function. Among the various post-translational modification reactions on proteins, the biosynthesis and processing of protein-bound N-linked oligosaccharides (N-glycans) is the most common. Using two-photon (2P)-excitation Förster resonance energy transfer (FRET)-fluorescence lifetime imaging microscopy (FLIM) we determined an average excited-state fluorescence lifetime of 2.2 ± 0.1 nanoseconds (ns) for the Golgi-resident N-glycan processing enzyme MNS1-G following FLIM measurements in the absence of an acceptor. Co-expression of MNS1-G and MNS1-R led to a significant quenching of the donor lifetime to an average of 1.9 ± 0.1 ns which indicates that the fluorophores of the analysed protein pair were close enough in Golgi membranes to undergo FRET and that MNS1-G likely interacts with MNS1-R. For GnTI-G, the average fluorescence lifetime was 2.2 ± 0.1 ns in the absence of the acceptor, but decreased to an average of 1.9 ± 0.1 ns in the presence of GnTI-R or MNS1-R (data not shown), indicating interactions between GnTI-G/GnTI-R and GnTI-G/MNS1-R. Altogether, the established FRET-FLIM data are consistent with previously performed co-IP data and we can conclude that at least the two tested N-glycan processing enzymes MNS1 and GnTI homodimerise and additionally form a GnTI/MNS1 heterodimer.



Golgi localization of full-length cis/medial-Golgi protein pairs in tobacco leaves.



In vivo 2P-FRET-FLIM analysis of full-length cis/medial-Golgi protein pairs.

Characterisation of small fluorescent protein iLOV for use in fluorescence lifetime imaging applications

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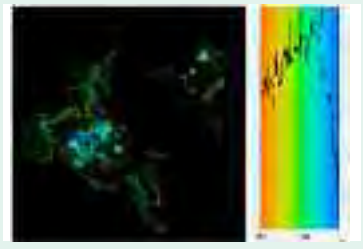
Our investigations aimed to test the potential of a small (~11kD) fluorescent protein, iLOV, as a marker for fluorescence lifetime imaging (FLIM) applications. Initial work has been conducted on a fusion of iLOV to the baculovirus protein P10, which has previously not been possible to successfully tag with EGFP. Insect cells grown in dishes infected with a recombinant version of Autographa californica nucleopolyhedrovirus (AcMNPV) encoding P10-iLOV, show formation of fluorescent tubular and aggregate type P10 structures. Using the FLIM system at CLF we have shown that iLOV has a long fluorescence lifetime (over 4ns) compared to GFP (~2.5ns). Our future plans are to test the protein in other cell systems and to explore the potential of iLOV as a partner in FRET-FLIM experiments to study protein interactions.



A fluorescence lifetime image of P10-iLOV structures in Trichoplusia ni (TN368) cells 96 hours post infection. The lifetimes shown here range from 3500-4500ps (3.5-4.5ns), p10-iLOV has an average fluorescence lifetime of ~4ns.

Ultrasensitive dark-field microspectroscopy with a super continuum source

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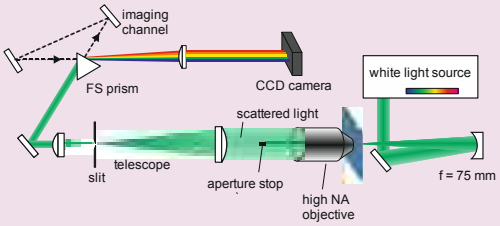
A highly sensitive dark-field microscope for visible scattering spectroscopy of nanoparticles has been developed. Front illumination with a curved mirror allows us to use 95% of the numerical aperture of the objective for detection. Subsequent losses are minimized by dispersing the scattered light with a prism.
In a first experiment, scattering spectra of single gold nanoparticles with an average diameter of 40 nm were measured. Variation in the total scattering cross section was correlated with the position of the resonance band, and was assigned to a distribution in particle size.
The efficient suppression of background light was demonstrated by the simultaneous measurement of the fluorescence of a single quantum dot and scattering from its surrounding. These results represent the first detection of the fluorescence of a single emitter without the use of a filter in the detection arm



Setup of the dark-field spectromicroscope

Imaging of Z-combretastatin induced apoptosis upon two-photon activation of E-combretastatin pro drugs

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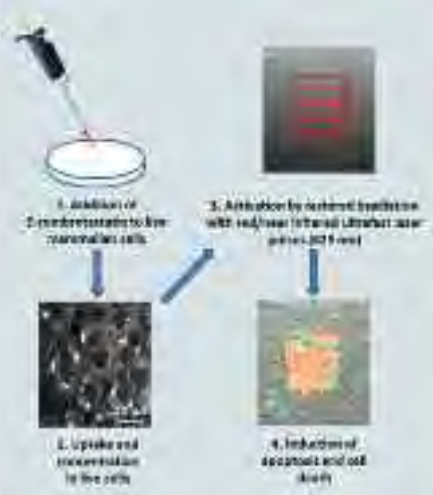
The aim of this research is to investigate E-combretastatins as safe photoactivatable pro-drugs for the treatment of cancer. It is proposed to use red or near-infrared pulsed laser light with better tissue penetration than UV to convert the pro-drug candidates to their highly active Z-isomers.

The cytotoxic (cell killing) effects of Z-combretastatins was determined by staining with markers for apoptosis (propidium iodide and AlexaFluor labelled annexin V) on a range of mammalian cell lines (CHO, HeLa, HUVEC). Monolayers of live CHO cells were incubated with the E-combretastatins, E-CA4 and E-CA4F, respectively for 2 h. Following incubation with the inactive pro-drug a small area (100 x 100 μm^2) was raster-scanned with the pulsed beam from a Ti-sapphire laser (625 nm) for 10 min, converting the E-combretastatin to the highly active Z-isomer. After 24 h the markers for apoptosis were added and selective cell killing in the irradiated areas observed.



Two-photon activation of E-combretastatins on monolayers of live CHO cells and cell killing. 1. The E-combretastatin was added to confluent live CHO cells at a concentration of 25 µM and 2. incubated for 30 min. 3. A pulsed laser beam (625 nm) was raster-scanned over an area of 100 μm^2 for 10 min (4.5 mW laser power). 4. Staining with propidium iodide and AlexaFluor-labelled annexin V showed cell killing by the activated pro-drug within 24 h.

Chemistry

Photodesorption and dissociation from a graphite surface

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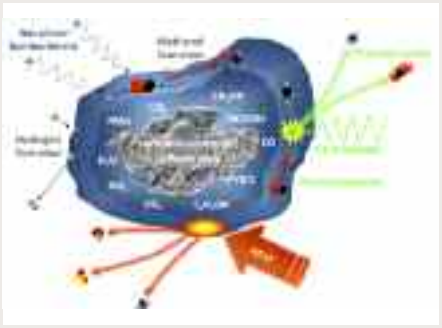
Ultraviolet (UV) processing of interstellar ices on the surface of carbonaceous and silicaceous grains gives rise to a range of molecules in the interstellar medium. Of particularly relevance in this respect are larger organic species such as glycolaldehyde and methyl formate, which have astrobiological significance. To investigate this UV processing, laser irradiation of model interstellar ices adsorbed on graphite was undertaken, with the aim of studying substrate mediated photochemistry. Laser induced desorption (LID) was observed for CO_2, CH_3OH, CH_3CH_2OH, HCO_2H, methyl formate and glycolaldehyde, adsorbed as pure ices and coadsorbed with H_2O ice. For CH_3OH, laser induced dissociation was also observed, giving CH_3 and OH fragments. The LID of methyl formate was strongly influenced by the presence of H_2O ice, with mixed ices showing less methyl formate LID. This result, and that for the dissociation of CH_3OH, are very significant for interstellar chemistry.



Processing of interstellar ices

The role of a heme pocket lysine residue during the geminate recombination of NO in the hemoprotein cytochrome c'

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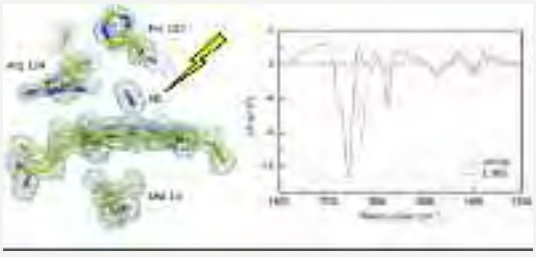
The ability of hemoproteins to discriminate between diatomic molecules, and the subsequent affinity for their chosen ligand, is fundamental to the existence of life. These binding processes are often controlled by precise structural arrangements in the protein, with heme pocket residues driving reactivity and specificity. One such protein, cytochrome c', has the ability to bind nitric oxide (NO) and carbon monoxide (CO) on opposite faces of the heme cofactor. The removal of a single distal residue (L16) allows NO to bind to the distal face of the heme cofactor, creating a 6c-NO species. By using ultrafast, time-resolved UV/visible and infra-red spectroscopy we have highlighted the kinetic and spectral (UV-vis and IR) differences for the geminate recombination of NO to either side of the heme cofactor and have shown that there is no escape of NO to the bulk solvent in an L16G variant. This work illustrates the importance of heme pocket architecture in modulating the control over heme-ligand reactivity and could have significant implications for other gas-binding proteins.



The heme environment in the crystal structures of ferrous, NO-bound cytochrome c' with key binding pocket residues shown (left). Time-resolved IR difference spectra for wild type and L16G cytochrome c' at 1 ps after NO photolysis.

Dynamics of chemical and photochemical reactions in solution

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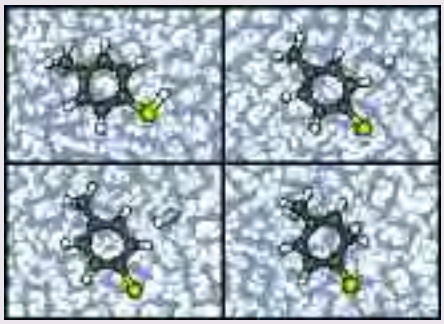
The liquid phase provides a medium for many important synthetic and biological chemical reactions, yet the role played by the solvent is often poorly defined. In a liquid environment, collisions between reactants and the solvent molecules occur with typical intervals of ~100 fs that are competitive with the timescales for chemical reaction. These solvent collisions influence the energy flow over the course of the reaction, equilibrate the products with the solvent bath, and may modify both the energy landscape over which reaction occurs and the motions of the reacting atoms and molecules (the reaction dynamics). Our investigations using ultrafast transient absorption spectroscopy seek to draw detailed comparisons between the dynamics of chemical reactions observed under isolated conditions in the gas phase, and those exhibited by the same reactions in liquids. Such contrasts provide robust evidence for the influence the solvent exerts on chemical mechanisms.



Snapshots of the short-time photodissociation dynamics of p-methylthiophenol in solution, showing H-atom loss and geminate recombination by H-atom addition to the ring.

Time-resolved vibrational spectroscopy of Trolox C photoionization

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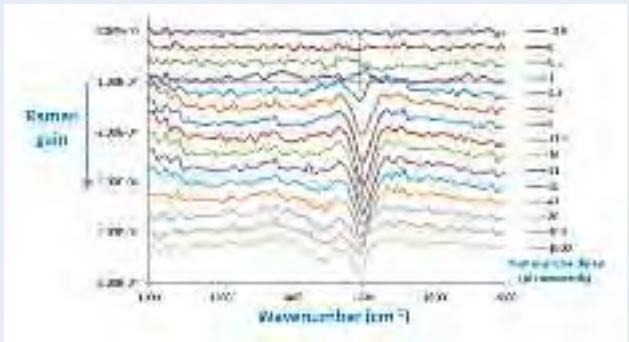
Trolox C has been widely used as a water soluble model compound for vitamin E, the important chain breaking antioxidant that protects humans against oxidative stress. Photoionization of Trolox C in solution represents an electron/proton transfer half-reaction in the repair of the chain-propagating peroxyl radical by the phenolic group of vitamin E. Together with UV-visible transient absorption spectroscopy, femtosecond stimulated resonance Raman spectroscopy (FSRS) and picosecond time-resolved infrared (TRIR) spectroscopy offer complementary views on the process of this reaction. FSRS offers both ultrafast kinetics and good spectral resolution but probes only certain vibrational modes of the radical products of the reaction. In contrast TRIR probes loss and regain of the ground state and a wider range of vibrational modes of both excited states and radical products. The results clearly demonstrate the two channels of excitation and ionization that depend on solvent.



Femtosecond stimulated Raman spectra from Trolox C in aqueous buffer at pH 7, obtained with actinic pulse at 266 nm and a Raman pump at 450 nm.

Intra- and intermolecular photoinduced electron transfer acceleration in azurin mutants labeled with a re-carbonyl-diimine chromophore

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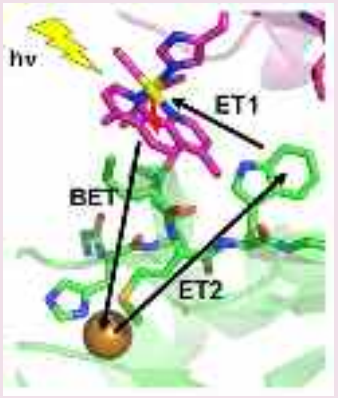
Long-range photoinduced electron transfer in proteins can be dramatically accelerated by inserting tryptophan into the electron-transfer pathway, which acts as an electron-hopping intermediate. This mechanism operates both intra- and intermolecularly in protein complexes. Time resolved IR spectroscopy investigated over a wide temporal range provides detailed understanding of the kinetics, as well as structural information on reactive excited states, intermediates and products.



Physics

Coherence studies of pulsed electron beams from point sources

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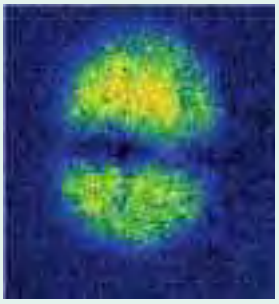
Electron microscopy and diffraction allow matter to be observed with far more acuity than with optical means, due to the much shorter wavelength. This technique already shows great promise for resolving complex bio-molecular structures such as proteins. Our recent studies have focused on developing a pulsed electron source, produced by focusing an ultrafast laser on a metal tip that comes to a point only tens of atoms across. Such electrons will facilitate time-resolved measurements whereby charge density becomes observable on atomic timescales. Work is ongoing to measure the transverse coherence length of electron beams from this source.



Measurements are being performed by means of electron holography, where an electron beam is split and recombined by a biprism. For a coherent beam this results in the generation of interference fringes, the properties of which allow the transverse coherence length to be determined. We have so far successfully demonstrated stable electron production from a nanotip and the operation of the biprism. We will soon demonstrate electron holography, allowing the coherence to be measured. We report the concept and design of this highly novel apparatus, our results to date and our immediate plans.

Ultrafast spectroscopy of plasmonic nanoantennas using the Pharos/Orpheus laser

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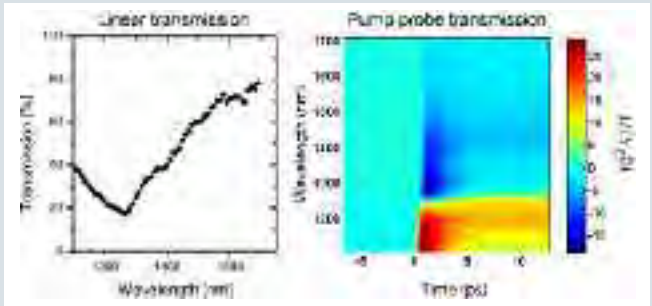
We investigate the femtosecond nonlinear optical response of plasmonic nanoantennas using a regenerative amplified laser system (Orpheus). Plasmonic nanoantennas were designed to provide a high local field enhancement with efficient coupling to far field radiation. These types of nanoantennas show a strong plasmonic response in the near-infrared.

We performed ultrafast pump-probe spectroscopy resulting in spectrally resolved maps of the ultrafast dynamics of the antenna array. A fast initial response is observed resulting from the excitation of hot electrons in the gold nanoantenna. The signal is consistent with a combination of transient bleaching and a redshift of the plasmon resonance.

Using the pump-probe maps, we will target specific materials of interest for interfacing with plasmonics for applications in nonlinear control and ultrafast switching.



Plasmonic antenna array (inset, scale bar 500nm) with transmission spectrum (left) and ultrafast pump-probe map (right) obtained using Pharos/Orpheus laser system.

Manipulation of a continuous beam of molecules by light pulses

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We report on the experimental observation of the action of multiple light pulses on the transverse motion of a continuous beam of fullerenes. The light potential is generated by non-resonant ultra-short laser pulses in perpendicular spatial overlap with the molecule beam. We observe a small but clear enhancement of the number of molecules in the central part of the molecular beam. Relatively low light intensity and short laser pulse duration prevent the molecule from fragmentation and ionization. Experimental results are in very good agreement by Monte Carlo trajectory simulations to explain the enhancement. This technique can prove to be useful for the focusing or collimation of large, neutral, and polarisable particles.