Biology

Photoacoustic spectroscopy

D. Birtill[1], A. Shah[1,2], M. Jaeger[1,2], A. Gertsch[1], J. Bamber[1]
([1]Joint Department of Physics)
([2]CRUK-EPSRC Cancer Imaging Centre, Institute of Cancer Research, UK)

D. Birtill
david.birtill@icr.ac.uk

A photoacoustic (PA) spectroscopy system has been built to study the differences between the PA spectra of oxygenated and deoxygenated blood, and various PA contrast agents, with a view to optimising the identification of these media in clinical PA images. A tunable laser delivers pulses (ns) of light into a sample causing it to momentarily expand and emit a pressure wave, the energy of which is measured by the computer using a digital oscilloscope that samples the signal from a focused 7.5 MHz ultrasound transducer. Once scanned at multiple wavelengths (400-700nm) the resulting optical spectra are corrected for some system variables, including the wavelength-dependent laser energy. The measured PA spectrum of oxygenated blood strongly resembles published optical absorption spectrum. Also the PA absorption spectrum of gold nano-particles compares well with standard spectrophotometer measurements. This system may have applications as a laboratory spectrophotometer for absorption spectra.

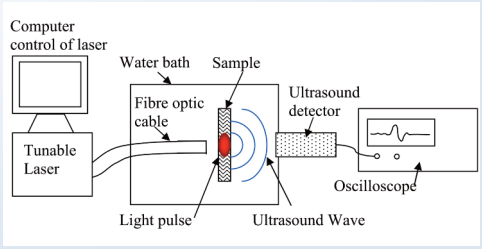


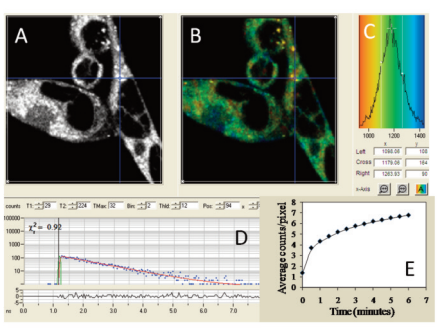
Diagram of the photoacoustic setup

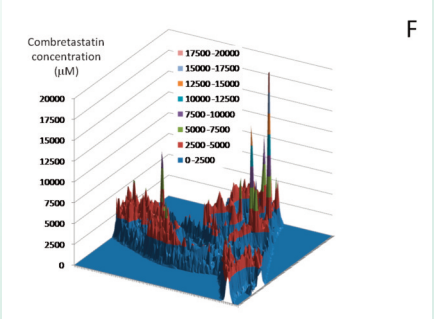
Two-photon excited fluorescence lifetime imaging of the intracellular uptake of (E)-combretastatin derivatives

R. H. Bisby, J. A. Hadfield, A. T. McGown, K. M. Scherer
(University of Salford, UK)
S. W. Botchway, A. W. Parker
(STFC, Rutherford Appleton Laboratory, UK)

R. H. Bisby
r.h.bisby@salford.ac.uk

Intracellular uptake by prostate cancer cells of the anticancer drug combretastatin A4 and analogues has been measured using multiphoton excited fluorescence lifetime imaging. Fluorescence from the less active trans isomers has been used to produce images (A) of the intracellular distribution of these compounds. The fluorescence lifetime image (B) shows a distribution of lifetimes (C, D) centred around 1.2 nanoseconds. Complementary studies in a range of solvents indicate that this fluorescence lifetime reports a viscous environment. Co-localisation studies with the lipid probe, nile red, suggest the drug is accumulated within cellular membranes and lipid droplets. Uptake of the combretastatin is rapid (E), occurring in minutes at room temperature. Combining fluorescence intensities and lifetimes shows that the intracellular concentrations (these are mapped within a field of cells (F)) exceed those in the surrounding medium by between 2 and 3 orders of magnitude, indicating accumulation is likely based on lipophilicity of the combretastatin molecule.





Two-photon fluorescence lifetime imaging of a fluorinated combretastatin in PC-3 cells. The intensity (A) and lifetime (B) images are shown after incubation with drug (10 μM) for 10 minutes. The lifetime distribution (C) is shown together with a single pixel fluorescence decay (D). The time-dependent increase in intracellular fluorescence intensity after addition of E-CA4F to the cells is shown in E. A map of intracellular concentration (in μM) is shown in F. (The images show a field of 70 x 70 μm)

The plant secretoryome: protein-protein interactions in the higher plant secretory pathway

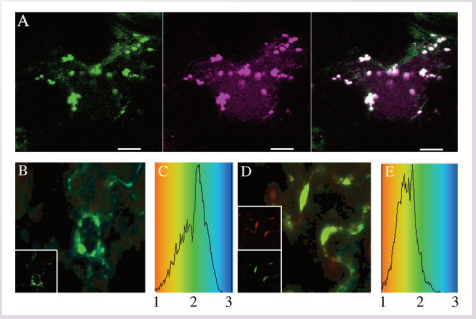
C. Hawes, I. Sparkes, A. Osterrieder, J. Schoberer
(Oxford Brookes University)
S. Botchway, and A. Ward
(STFC, Rutherford Appleton Laboratory, UK)

C. Hawes
chawes@brookes.ac.uk

We have been continuing to look at protein-protein interactions in the plant Golgi apparatus, concentrating on interactions between glycosyl transferases in the cisternal membranes of the Golgi stack. Initial results from FLIM data indicate that FRET can occur between fluorescent proteins tagged to a range of cis-Golgi enzymes. Our data suggests for the first time homo- and hetero-oligomerisation between plant Golgi processing enzymes.

In a new project with a collaborator from INRA, Nantes, we have been looking at the formation of wheat storage protein bodies in tobacco cells. FRET-FLIM (Figure) was used to show the interaction between N- and C- terminal domains of the storage proteins γ-gliadin, thus confirming the confocal and biochemical data from expression of the protein in tobacco.

During the year we obtained the first data from a newly constructed TIRF-based laser trapping microscope and successfully captured Golgi bodies targeted with fluorescent proteins expressed in tobacco leaves. The instrument will be used for a study of the interactions of the plant Golgi apparatus with the endoplasmic reticulum and cytoskeleton.



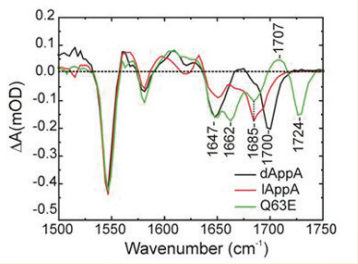
A .Expression of the C terminal domain ofγ-gliadin fused to-GFP and N terminal domainfused to RFP in tobacco leaves showing co-localisation of the protein bodies. B-F. FRET-FLIM analysis in tobacco leaf epidermal cells. Lifetime images of Cter-γ-gliadin-GFP (B), and Cter-γ-gliadin-GFP in a cell coexpressing Nter-γ-gliadin-mRFP (D) are shown. Insets are confocal images showing expression of Nter-γ-gliadin-mRFP (red) and or Cter-γ-gliadin-GFP (green) in the cells shown. C,E Curves of B & E showing reduction in GFP lifetime in co-expressing cells.

Probing the mechanism of blue light sensing BLUF domain proteins: A Study through transient infra-red spectroscopy, isotope editing and mutagenesis

A. Lukacs, R-K. Zhao, S. R. Meech
(University of East Anglia)
R. Brust, A. Haigney, P. J. Tonge
(Stony Brook University, USA)
G. M. Greetham, M. Towrie
(STFC, Rutherford Appleton Laboratory, UK)

S. R. Meech
s.meech@uea.ac.uk

Flavoproteins are a large family of proteins that contain a covalently or non-covalently bound flavin cofactor. Although the isoalloxazine ring of the flavin functions predominantly as an electron transfer intermediate in biochemical oxidation-reduction reactions, there are at least three subfamilies of flavoproteins that function as photoreceptors: the light-oxygen-voltage (LOV) domain proteins, the cryptochromes, and the Blue Light Using FAD (BLUF)-domain proteins.The chromophores in other well known photoreceptors such as the rhodopsins, xanthopsins and phytochromes undergo an isomerization when light is absorbed. However, this is not the case for flavoprotein receptors. Consequently there is substantial interest in understanding how absorption of light by the flavin is coupled to the conformational change(s) that lead to the signaling state. In the LOV domain proteins a cysteinyl-flavin adduct is transiently formed in the signaling state. However, in the cryptochromes and BLUF proteins, the initial structural changes resulting from photoexcitation are less well established.



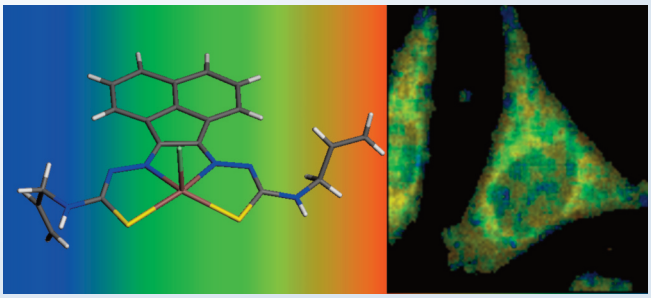
TRIR spectra of dAppA$_{BLUF}$ (black), lAppABLUF (red) and Q63E AppA$_{BLUF}$ (green) bound to FAD. Protein concentration was 2 mM in pD 8 phosphate buffer and the TRIR spectra were recorded with a time delay of 3 ps. The spectra have been normalized to the intense FAD ring bleach mode at 1547 cm^{-1}.

Kinetically stable metal complexes for multimodality PET/SPECT and optical fluorescence microscopy probed in vitro by FLIM

J. R. Dilworth, S. Faulkner M. W. Jones and P. A. Waghorn
(University of Oxford, UK)
S. I. Pascu and R. Arrowsmith
(University of Bath, UK)
S. W. Botchway and A. Parker
(STFC, Rutherford Appleton Laboratory, UK)

S. I. Pascu
s.pascu@bath.ac.uk

Molecular imaging is a rapidly expanding field of global importance for both the diagnosis and personalised therapy of a range of disease states. There is an increasing demand for molecular probes that can be used for imaging and early detection of specific cancers which represent major life risks worldwide, such as breast, colon, and prostate cancer. Shortage of oxygen (hypoxia) is a common cause of cancer treatment failure: reliable imaging tools for tumour hypoxia would be invaluable in planning treatment regimens and predicting clinical outcomes. Confocal fluorescence microscopy has been used extensively to track compounds and follow processes in cells. We have designed and tested in vitro a series of kinetically stable fluorescent metal complexes, that can be used for whole body imaging using gamma or positron emission and may be monitored in cells by virtue of their 1 or 2-photon excited fluorescence and fluorescence lifetime imaging (FLIM).



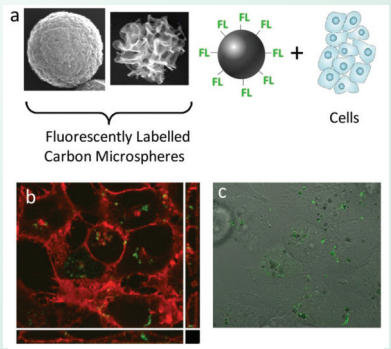
Two–Photon Fluorescence lifetime imaging map (λ_{ex} 910 nm, 20 min incubation) in prostate cancer cells for a new gallium aromatic complex with relevance to positron emission tomography of cancer cells

Porous carbon microspheres: Solution phenomena and cellular uptake

S. J. Quinn, L. M. Magno,
(University College Dublin, Ireland)
P. E. Colavita, P. Duffy
(Trinity College Dublin, Ireland)
R. Yadav, S. K. Roberts, A. D. Ward,
S. W. Botchway
(STFC, Rutherford Appleton Laboratory, UK)

S. Quinn
susan.quinn@ucd.ie

Carbon based micro and nanomaterials are attractive for biological applications because of their excellent biocompatibility profile. Porous microparticles, prepared via a routine synthesis, are easy to handle and purify, and combine a high specific surface area with carbon's high functionality. We wish to leverage these properties for the purposes of cell imaging and delivery. In this work, porous carbon microspheres with a high specific surface area were prepared and their fundamental properties studied using Raman optical tweezers. These experiments showed that 532 nm excitation of microspheres trapped in solvents that display poor heat conduction resulted in graphitization and incandescence. These phenomena were also observed for microspheres in the water presence of a cationic lipid (DOTAP). The uptake of the particles by cells was demonstrated by fluorescence confocal microscopy imaging of fluorescently labelled microspheres. Finally, the ability to leverage their optical absorptivity in order to cause carbon graphitization and cell death was investigated.



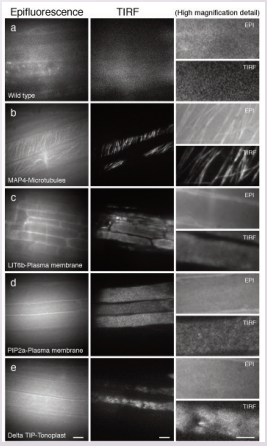
Uptake of fluorescently labelled carbon microspheres by HeLa cells. Confocal images of HeLa cells after incubation with FL-LiDCA particles. Z-scan rendering of carbon particle internalization in HeLa cells; the cell membrane is stained with DiI. and Transmission image showing fluorescent nanoparticles, shown below.

Total internal reflection and single molecule fluorescence microscopy in plant cells

G. Vizcay-Barrena, Z. A. Wilson
(University of Nottingham, UK)
S. E. D. Webb, M. L. Martin-Fernandez
(STFC, Rutherford Appleton Laboratory, UK)

S. E. D. Webb
stephen.webb@stfc.ac.uk

Total internal reflection fluorescence microscopy (TIRFM) has been proven to be an extremely powerful technique in animal cell research for generating high contrast images and dynamic protein conformation information. However, there has long been a perception that TIRF is not feasible in plant cells because the cell wall would restrict the penetration of the evanescent field and lead to scattering of illumination. By comparative analysis of epifluorescence and TIRF in root cells, we demonstrate for the first time that TIRF can generate high contrast images, superior to other approaches from intact plant cells. We also show that TIRF imaging is not only possible at the plasma membrane level, but also in subcellular organelles, for example the nucleus, due to the presence of the central vacuole. Importantly, we demonstrate that this is TIRF excitation, and not TIRF-like excitation described as variable-angle epifluorescence microscopy (VAEM) and show how to distinguish the two techniques in practical microscopy. These TIRF images show the highest signal-to-background ratio and we show that they can be used for single molecule microscopy. Rare protein events, which would otherwise be masked by the average molecular behaviour, can therefore be detected, including the conformations and oligomerisation states of interacting proteins and signalling networks in vivo. The demonstration of the application of TIRFM and single-molecule analysis to plant cells therefore opens up a new range of possibilities for plant cell imaging.



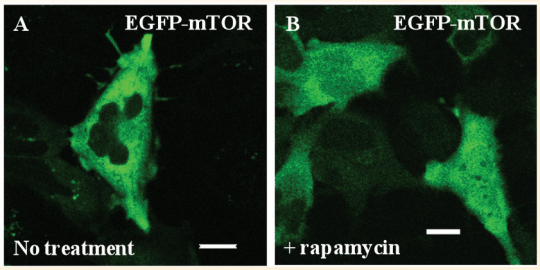
Analysis of plasma membrane and cytoskeleton markers by Epifluorescence and TIRF microscopy in roots.

Rapamycin does not affect the interaction between mTOR and raptor but causes increased nuclear levels of highly expressed mTOR in HeLa cells

R. B. Yadav, C. D. Stubbs, A. W. Parker, S. W. Botchway
(STFC, Rutherford Appleton Laboratory, UK)
V. Iadevaia, C. G. Proud
(School of Biological Sciences)

C. D. Stubbs
chris.stubbs@stfc.ac.uk

We have used confocal microscopy and FRET-FLIM to investigate the localisation and protein interactions involved within the mammalian Target of Rapamycin (mTOR) signaling pathway. mTOR protein kinase is a central regulator of cellular growth, metabolism and proliferation in all eukaryotes. The mTOR pathway is deregulated in many human diseases such as cancer and type 2 diabetes. Rapamycin, an anticancer drug used to inhibit mTOR, is in clinical trials for cancer treatment but as yet how exactly rapamycin perturbs the functions of mTOR is not completely understood. In the present study we have investigated the localisation of mTOR and its molecular interaction with raptor using FRET-FLIM technology in live HeLa cells (an immortal cell line derived from cervical cancer cells). Effect of rapamycin on the localisation of mTOR and its interaction with raptor was investigated. It was found that rapamycin did not dissociate the mTOR-raptor interaction.



Rapamycin treatment affects the localisation of EGFP-mTOR in HeLa cells. Confocal images of HeLa cells expressing EGFP-mTOR A) no treatment and B) rapamycin (100 nM) treatment for 24 h.

Chemistry

LIAD-fs: A novel method for studies of neutral biomolecules in the gas phase

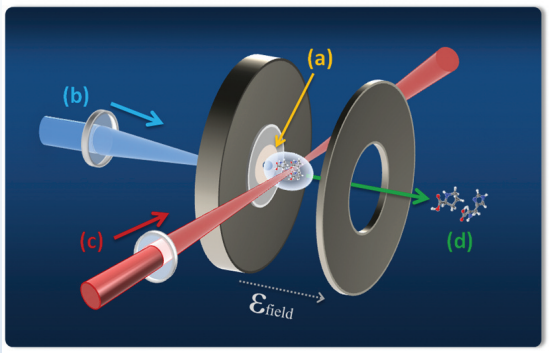
C. R. Calvert, O. Kelly, L. Belshaw, M. J. Duffy, R. B. King, I. D. Williams and J. B. Greenwood
(Queen's University Belfast)
T. J. Kelly and J. T. Costello
(Dublin City University)

C. R. Calvert
c.calvert@qub.ac.uk

Intense femtosecond laser pulses provide unique tools for influencing and observing molecular dynamics on ultrashort timescales.

We report here on recent experimental studies of femtosecond laser interactions with molecules of biological interest, using the UFL2 laser from the EPSRC laser loan pool. In a novel experimental approach we have adopted Laser Induced Acoustic Desorption to produce a gas phase target of neutral molecules, which are then exposed to an fs laser pulse, with subsequent ionisation/fragmentation products being mass-analysed in our KEIRA ion trap.

Using critical laser pulse parameters, such as intensity and pulse duration, we have demonstrated control over the molecular fragmentation and ionisation processes for a range of amino acids, small peptides and DNA bases. This technique has provided insight into the dynamics of such biomolecules in intense laser fields and provides a valuable platform for future studies where ultrashort pulses promise to enable observation and control of ultrafast processes in biomolecules.



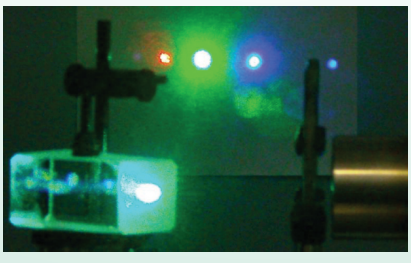
Schematic of LIAD-fs technique for studying biomolecules in the gas phase. The sample is deposited on a Ta foil (a) which is then back irradiated using a UV ns pulse (b). A femtosecond laser (c) is then interacted with the resulting biomolecular plume, with charged products extracted and analysed electrostatically (d).

Photoacoustic stimulated raman spectroscopy (PARS) for trace detection of molecular hydrogen

C. L. Spencer, V. Watson and M. Hippler
(University of Sheffield, UK)

M. Hippler
m.hippler@sheffield.ac.uk

Sensitive and selective detection of molecular hydrogen (H_2) is very relevant, for example for a future hydrogen economy (use of hydrogen gas as fuel), or to detect traces of H_2 in metallurgy (H_2 can compromise steel enclosures). Spectroscopic detection of H_2, however, is notoriously difficult to achieve.
We have set up two different schemes of stimulated Raman photoacoustic detection of H_2, one with a Raman shifter and a second scheme with a tuneable dye laser, to generate stimulating Raman excitation. In addition, green Nd:YAG light served as Raman pump radiation. Both beams were focused into a cell with a gas mixture to be analysed. H_2 absorbed radiation due to the stimulated Raman effect, and photoacoustic signals were picked up by a microphone.



Photograph showing the red Stokes, green Rayleigh, and blue/UV anti-Stokes radiation produced from the H_2 Raman shifter, dispersed by a prism.

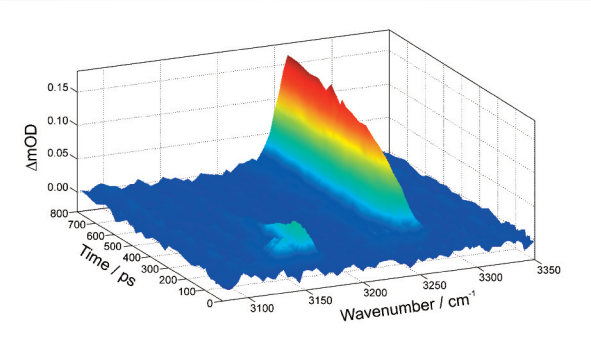
Excellent detection linearity and detection limits in the ppm range for H_2 in 1 atm air were achieved. The schemes are thus suitable for reliable detection of H_2 in ambient air and gas mixtures.

Dynamics of chemical and photochemical reactions in solution

S. J. Greaves, R. A. Rose, T. A. A. Oliver, M. N. R. Ashfold, A. J. Orr-Ewing
(University of Bristol, UK)
M. Towrie, G. M. Greetham, I. P. Clark
(STFC, Rutherford Appleton Laboratory, UK)

A. Orr-Ewing
a.orr-Ewing@bris.ac.uk

Transient broadband IR absorption spectra have been obtained of the HCN products of bimolecular reactions of CN radicals with cyclohexane in solution in various chlorinated organic solvents. The spectra reveal that the HCN is initially formed with vibrational excitation in both its C–H stretching and bending vibrational modes, and that this mode-specific vibrational excitation relaxes through coupling to the solvent with solvent-dependent time constants of 130 - 270 ps. The early time reaction dynamics are remarkably similar to those previously reported for gas-phase reactions of CN radicals with hydrocarbons, despite the presence of a solvent. Complementary dynamical calculations have been performed that, when combined with the experimental results, provide acute insights concerning the influence of the solvent on the fundamental mechanisms of chemical reactions in solution.



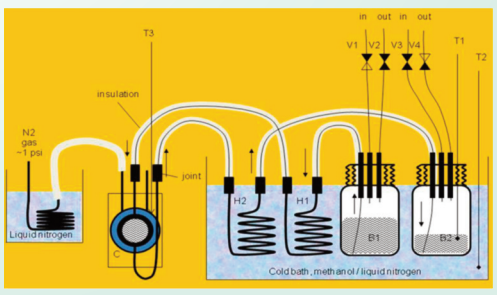
Picosecond time-resolved infrared spectroscopy of arylpentazole

P. Portius, M. Davis
(University of Sheffield, UK)
M. Towrie
(STFC, Rutherford Appleton Laboratory, UK)

P. Portius
p.portius@sheffield.ac.uk

Nitrogen-rich molecules are important for chemical energy storage and have a major advantage over conventional solutions to the problem of chemical energy storage, since their decomposition releases almost exclusively the environmentally friendly dinitrogen.[1] In search for stable compounds with high nitrogen content, methods have to be found which allow the introduction of nitrogen in synthetic procedures. The most relevant of these have traditionally been the coordination of the azido anion (N_3^-) to main group elements[2] or the derivatisation of tetrazoles[1, 3] (R-CN_4-R'). Meanwhile, the scope of these methods has been largely realized and forms the basis of various successful applications.

[1] Steinhauser, G.; Klapoetke, T. M., Angew. Chem. Int. Ed. 2008, 47, 2-20.
[2] a) Portius, P.; Filippou, A. C.; Schnakenburg, G.; Davis, M.; Wehrstedt, K.-D., Angew. Chem. Int. Ed. 2010, 49, 8013-8016; b) Mueller, J., Coord. Chem. Rev. 2002, 235, 105-119; c) Banert, K.; Joo, Y.-H.; Ruffer, T.; Walfort, B.; Lang, H., Angew. Chem. 2007, 119, 1187-1190.
[3] a) Singh, R. P.; Verma, R. D.; Meshri, D. T.; Shreeve, J.M., Angew. Chem. Int. Ed. 2006, 45, 3584-3601; b) Stierstorfer, J.; Tarantik, K. R.; Klapoetke, T. M., Chemistry--A European Journal 2009, 15, 5775-5792.

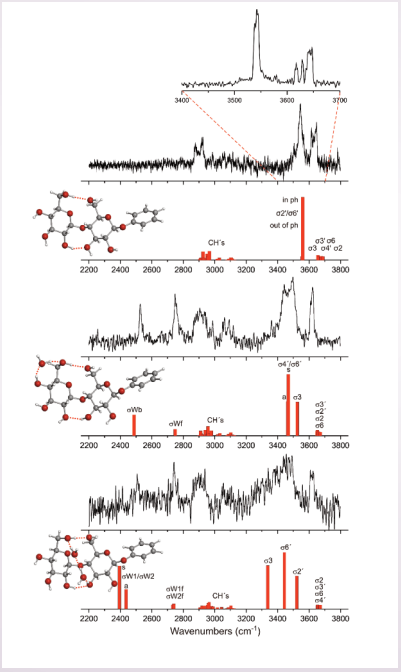


Design of the flow system for low temperature picosecond-TRIR as integrated in the ULTRA facility; V1-V4 solenoid valves applying N_2 pressure (0.3 bar) or releasing pressure from the reservoir bottles B1 and B2, H1 and H2 heat exchange coils, T1-T3 thermocouples, C commercial variable temperature spectroscopic cell. The arrows indicate the direction of flow when V1(in)&V4(out) are open and V2&V3 closed.

Isotopic hydration of cellobiose: vibrational spectroscopy and dynamical simulations

J. P. Simons, N. Mayorkas
(University of Oxford, UK)
B. G. Davis
(Chemistry Research Laboratory, UK)
E. . Cocinero
(University of Bilbao, Spain)
M. Pincu, R. B. Gerber
(University of California, USA)
B. Brauer
(The Hebrew University, Israel)

J. Simons
John.Simons@chem.ox.ac.uk



Experimental and (DFT) computed IR spectra and the corresponding hydrogen-bonded molecular structures of phenyl β cellobioside (top), phenyl β cellobioside•D_2O (middle) and phenyl β cellobioside•$(D_2O)_2$(bottom).

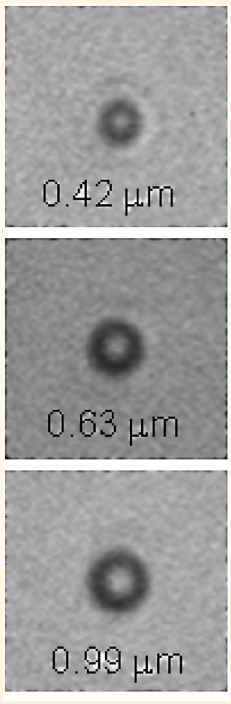
Interactions with water can influence both the conformation and the bio-activity of carbohydrates. If sugars didn't change shape, life would be radically different and some, perhaps many, biological processes just would not work anymore. The factors which dictate the conformation of cellobiose, the basic building block of cellulose, remain something of an enigma. To address this issue, water molecules have been added to a range of 'natural' carbohydrates, including cellobiose , under controlled conditions in a cold molecular beam and their hydrated molecular structures have been interrogated through infrared laser spectroscopy. A new and original 'twist' to this approach has been introduced by using heavy water (D_2O) which allows the infrared spectra associated with the carbohydrate (OH modes) and its hydration shell (OD modes) to be separately identified, analysed and interpreted.

Optical trapping of sub-micron liquid aerosol droplets

A. Ward
(STFC, Rutherford Appleton Laboratory,UK)
M. King
(University of London, UK)

A.Ward
andy.ward@stfc.ac.uk

Laser tweezers have become increasingly popular in the study of atmospheric aerosol science because of the unique way that individual micron-scale aerosol droplets can be trapped and retained for periods of several hours. Recent work has highlighted that conventional single-beam gradient optical trapping becomes unstable when the particle diameter is reduced to below 2 microns. This finding has a significant impact, for aerosol science, as sub-micron aerosol particulates are abundant in the atmosphere. Using a counter-propagating laser trapping configuration, particles can be captured with sizes from about 0.4 μm to more than 6 μm. Alternative methods for characterizing particle size are discussed and initial studies into a Raman spectra based technique show promise and indicate that sizing of sub-micron aerosol droplets is realistic. The extension in the trapping diameter range for aerosol particles will have application in determining atmospheric aerosol reaction and light scattering properties.



Examples of droplet images in optical focus when using the counter-propagating laser tweezers configuration

Physics

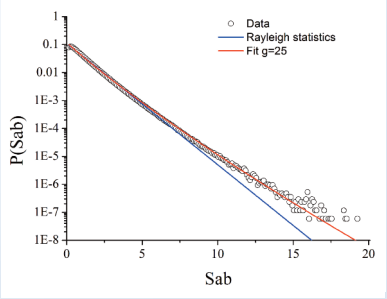
Ultrafast manipulation of photon transport and molecular beams

T. Strudley, P. Venn, H. Ulbricht, O. L. Muskens
(University of Southampton, UK)

O. L. Muskens
o.muskens@soton.ac.uk

We used a fs-pulsed laser for two different experiments opening new avenues towards the ultrafast manipulation of massive particles in gas-phase beams and of the mesoscopic transport of light. We demonstrated for the first time a mechanical focusing effect by off-resonant dipole interaction acting on the centre of mass motion of large neutral molecules such as tetra-phenyl-porphyrin (TPP). Focusing is enabled by interactions of a single molecule with many weak light pulses.

The mesoscopic transport of light through a random media consisting of a layer of very strongly scattering GaP nanowires in which the mean free path is much shorter than the depth of the layer was also investigated. The statistical distribution of transmitted intensities is compared to the normal Rayleigh distribution in which there are an infinite number of transmission modes. A significant decrease in the number of independent transmission modes to 25 can be seen in the figure.



Distribution of angular intensities normalised to ensemble average (Sab).