Imaging and dynamics for physical and life sciences

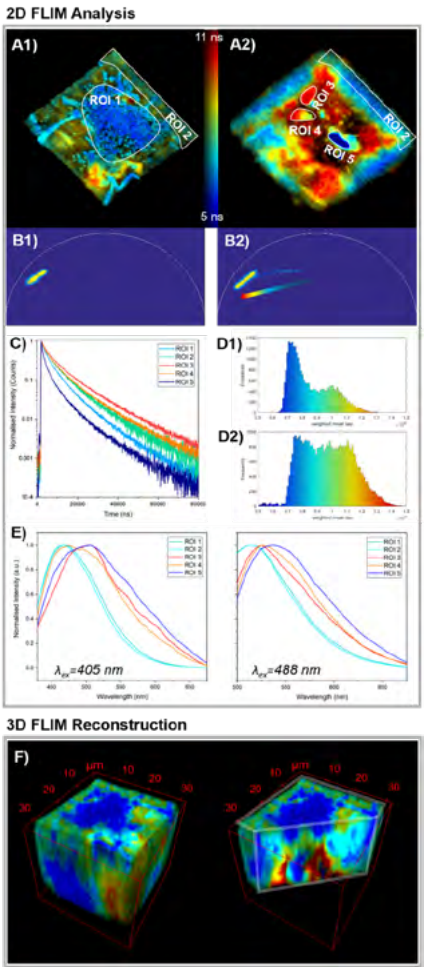
Multimodal imaging of autofluorescent sites reveals varied chemical speciation in zeolites for catalysis

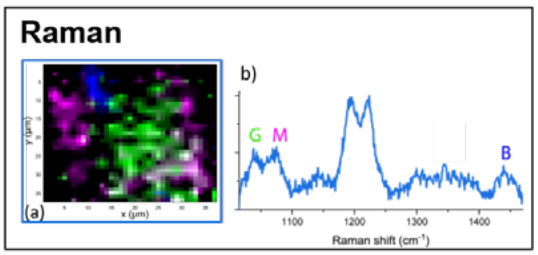
N.E. Omori, I. Lezcano-Gonzalez, A.G. Greenaway, A.M. Beale *(Department of Chemistry, University College London, UK; Research Complex at Harwell, STFC Rutherford Appleton Laboratory, Harwell Campus, Didcot, UK)*
A. Candeo, S. Mosca *(Central Laser Facility, Research Complex at Harwell, STFC Rutherford Appleton Laboratory, Harwell Campus, Didcot, UK),*
I.K. Robinson *(London Centre for Nanotechnology, University College London, UK; Brookhaven National Laboratory, Upton, New York, USA)*
L. Li *(Advanced Photon Source, Argonne National Laboratory, Lemont, Illinois, USA)*
P. Collier (Johnson Matthey Technology Centre, Reading UK)

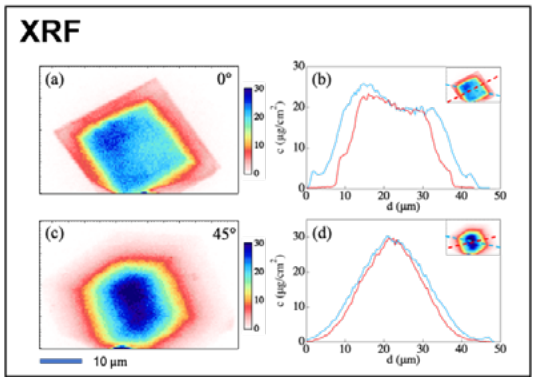
A multimodal imaging study of chabazite is used to show the distribution of and discriminate between different emissive deposits arising as a result of the detemplation process. 3D confocal imaging, fluorescence lifetime imaging, spectral imaging, and Raman mapping are used to show three different types of emissive behaviours each characterised by different spatial distributions, trends in lifetime, spectral signals, and Raman signatures. A notable difference is seen in the morphology of agglomerated surface deposits and larger subsurface deposits, which experience lifetime augmentation due to spatial confinement. The distribution of organic residue throughout the crystal volume is comparable to XRF mapping that shows Si enrichment on the outer edges and higher Al content through the centre, demonstrating that the technique can also be used to indirectly comment on the compositional chemistry of the inorganic framework.

Reproduced from Omori, N. et al. Multimodal Imaging of Autofluorescent Sites Reveals Varied Chemical Speciation in SSZ-13 Crystals. Angew. Chem. Int. Ed. 2021, 60, 5125 – 5131, © 2020 Wiley-VCH GmbH. doi:10.1002/anie.202015016

Contact: A.M. Beale (andrew.beale@ucl.ac.uk)







Left: FLIM ensemble of chabazite calcined at 550°C acquired at λ_{ex} = 488 nm including the A) map of weighted average lifetimes (1) for the uppermost plane and (2) for the central plane, B) phasor plots, C) fluorescence decays for selected ROIs, D) frequency histograms of lifetimes, and E) spectra for ROIs acquired at λ_{ex}= 405 nm and 488 nm.

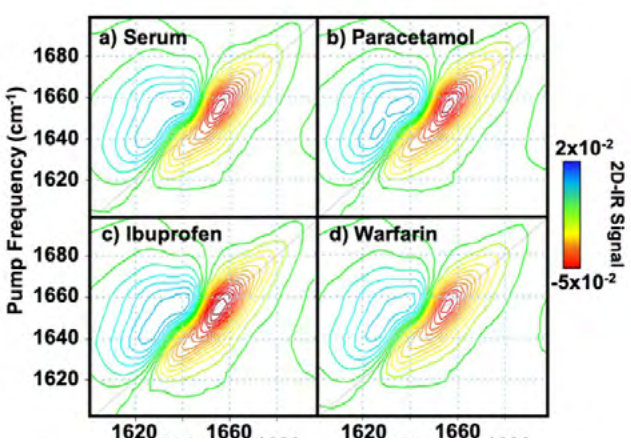
Right: Raman maps showing the distribution of selected vibrations for chabazite calcined at 550°C (top) and XRF tomography of wet-ion exchanged Cu-SSZ-13 crystals obtained at the Argonne National Laboratory with 10 keV photons (bottom) where (a) & (c) are the projected Cu maps at 0º and 45º to the crystal and (b) & (d) are the respective line profiles.

Detection of drug-protein complexes in aqueous serum using two-dimensional infrared spectroscopy

S.H. Rutherford *(Department of Physics, University of Strathclyde, Glasgow, UK)*
G.M. Greetham, M. Towrie, A.W. Parker *(Central Laser Facility, STFC Rutherford Appleton Laboratory, Harwell Campus, Didcot, UK)*
M.J. Baker *(Department of Chemistry, University of Strathclyde, Glasgow, UK)*
N.T. Hunt *(Department of Chemistry, University of York, UK)*

This report demonstrates proof of concept for the use of 2D-IR spectroscopy to detect drug binding to the serum albumin protein in blood serum. 2D-IR spectroscopy of blood serum to which the common drug molecules paracetamol, ibuprofen and warfarin have been added showed characteristic changes in the amide I region due to binding of the drugs to serum albumin. These results will motivate further work to clarify limits of detection and to assign the structural origins of the changes observed definitively. The demonstrated ability to measure different protein structural responses due to binding of drugs at physiological concentrations, however, suggests that the 2D-IR method has the potential to be used for rapid screening of protein-drug combinations or for monitoring of serum concentrations of specific drugs.

Contact: N.T. Hunt (neil.hunt@york.ac.uk)



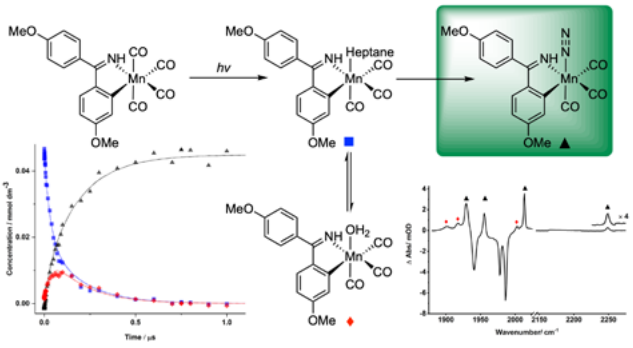
2D-IR spectra of a) serum, and serum spiked with b) paracetamol 2:1, c) ibuprofen 2:1 and d) warfarin 0.07:1. All spectra are plotted using the same scale, see colour bar.

Identification of a rare manganese dinitrogen complex

J.B. Eastwood, L.A. Hammarback, M.T. McRobie, I.J.S. Fairlamb, J.M. Lynam *(Department of Chemistry, University of York, UK)*
I.P. Clark, M. Towrie *(Central Laser Facility, STFC Rutherford Appleton Laboratory, Harwell Campus, Didcot, UK)*

A novel manganese dinitrogen complex has been identified by time-resolved infra-red spectroscopy. Light-induced loss of a carbonyl ligand from a stable precursor results in a highly reactive solvent complex. Displacement of the weakly held solvent then permits binding of N_2 to the manganese. The presence of the dinitrogen ligand was identified with the aid of isotopically enriched $^{15}N_2$ inducing the predicted shift in the MnN≡N vibrational mode. Kinetic simulations revealed the nature of competitive N_2 and H_2O binding to the metal and allowed for the rate constants for solvent substitution to be determined.

Contact: J.M. Lynam (jason.lynam@york.ac.uk)

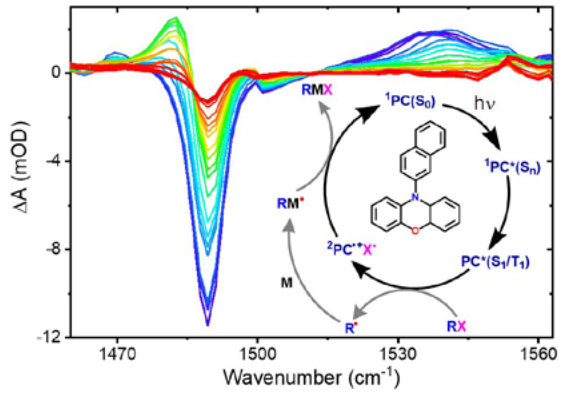


A new manganese-containing dinitrogen complex has been identified

Mapping photochemical cycles over femtosecond to millisecond timescales using transient absorption spectroscopy

L. Lewis-Borrell, M. Sneha, A. Bhattacherjee, A.J. Orr-Ewing *(School of Chemistry, University of Bristol, UK)*

Activation of chemical reactions using visible or ultraviolet light is a promising route to the sustainable synthesis of important compounds and materials such as pharmaceuticals or polymers. However, the mechanisms of such reactions follow complicated, multi-step pathways and the chemistry can be difficult to control. Systematic study of the short-lived reactive intermediates, their lifetimes, and their preferred reactions at each step along the reaction path using time-resolved infra-red spectroscopy offers new opportunities to understand and harness this light-activated chemistry.



Time-resolved infra-red spectra of a UV-excited organic photocatalyst used to drive photoredox reaction cycles of the type shown.

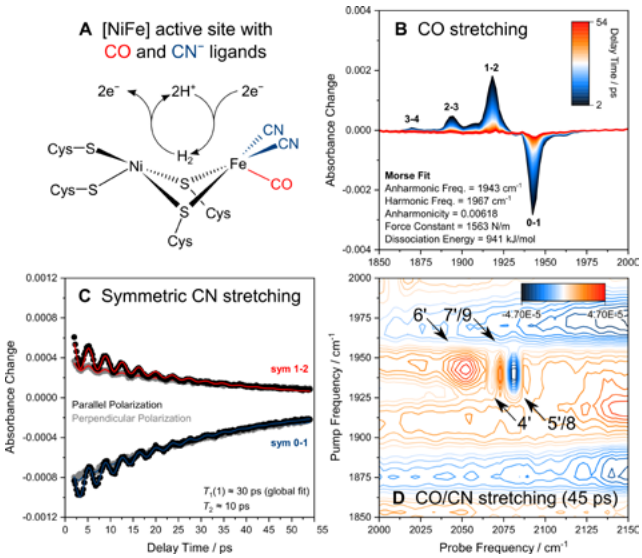
Contact: A.J. Orr-Ewing (a.orr-ewing@bristol.ac.uk)

Understanding hydrogenases by ultrafast and 2D-IR spectroscopy: proof-of-concept, recent advances and future avenues

M. Horch *(Department of Physics, Freie Universität Berlin, Germany; Department of Chemistry & York Biomedical Research Institute, University of York, UK; Department of Chemistry, Technische Universität Berlin, Germany)*
N.T. Hunt, A. Parkin, B. Procacci, J. Walton, S.L.D. Wrathall *(Department of Chemistry & York Biomedical Research Institute, University of York, UK)*
G.M. Greetham *(Central Laser Facility, STFC Rutherford Appleton Laboratory, Harwell Campus, Didcot, UK)*
A. Al-Shameri, C.J. Kulka, L. Lauterbach, O. Lenz, C. Lorent, J. Schoknecht, C. Schulz, I. Zebger *(Department of Chemistry, Technische Universität Berlin, Germany)*
J.A. Birrell, W. Lubitz, E.J. Reijerse *(Max Planck Institute for Chemical Energy Conversion, Mülheim an der Ruhr, Germany)*
P. Rodríguez Maciá *(Department of Chemistry University of Oxford, UK; Max Planck Institute for Chemical Energy Conversion, Mülheim an der Ruhr, Germany)*

Utilizing active-site CO and CN^- ligands as vibrational reporter groups, the structure, function, and dynamics of hydrogen-transforming biocatalysts, so-called hydrogenases, have been investigated by ultrafast infrared (IR) pump-probe and two-dimensional (2D) IR spectroscopies. Using these techniques, detailed insights into bond properties of the catalytically relevant CO ligand were obtained. In addition, fast relaxation rates of the CO and two CN stretching modes revealed highly efficient energy transfer – both from the catalytic metal site towards the protein matrix and between the two sets of ligands. Coupling between all three CX ligand vibrations could also be inferred from 2D-IR cross peaks and quantum beats observed in time series of IR pump-probe spectra. Both features allow assigning catalytic intermediates in complex reaction mixtures. Furthermore, analysis of the quantum beat decay revealed the time and length scales of active-site equilibrium dynamics, highlighting the relevance of entatic states in biological hydrogen transformation.

Reproduced from https://doi.org/10.1039/C9SC02851J with permission from the Royal Society of Chemistry



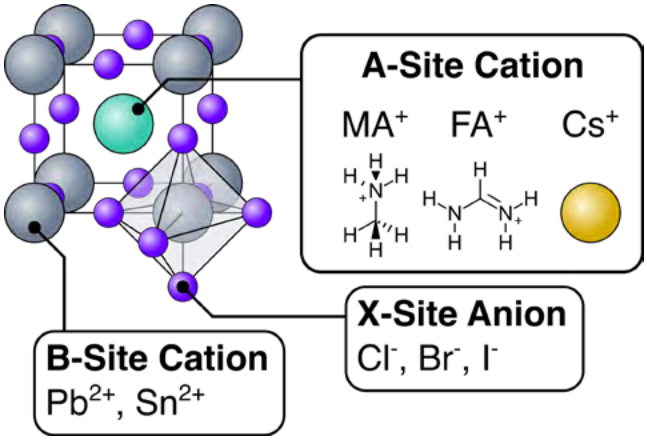
(A) Active site structure of [NiFe] hydrogenases. Diatomic ligands used as IR reporter groups are highlighted. (B) CO stretch transitions observed by ultrafast IR pump-probe spectroscopy and extracted bond properties. (C) Population and coherence decay observed for coupled CN stretch modes by ultrafast IR pump-probe spectroscopy. Time traces reflect the symmetric CN stretch mode. Extracted quantities are indicated. (D) Cross peaks observed by 2D-IR-spectroscopy, reflecting energy transfer between CO and CN stretch modes.

Contact: M. Horch (marius.horch@fu-berlin.de)

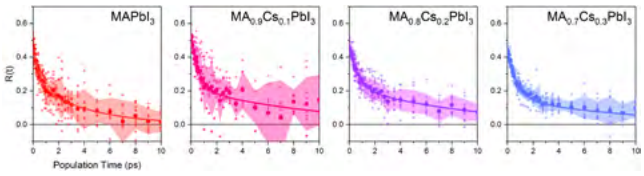
Understanding cation dynamics in caesium substituted organohalide perovskites

N.P. Gallop, A.A. Bakulin *(Department of Chemistry, Imperial College London, Molecular Sciences Research Hub, London, UK)*
J. Ye, R. Hoye *(Department of Materials, Imperial College London, Royal School of Mines, London, UK)*
G. Greetham *(Central Laser Facility, STFC Rutherford Appleton Laboratory, Harwell Campus, Didcot, UK)*

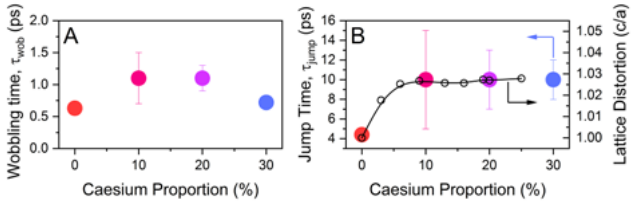
In this work, we aim to understand the effect of caesium substitution on the cation dynamics of organohalide perovskites (OHPs). This family of materials show immense promise as next-generation solar cells; however, there are still many aspects to them that we do not entirely understand. Amongst these is a complete understanding of the dynamics of the organic cations within the cell. To this end, we use 2DIR transient anisotropy measurements to probe the dynamics of methylammonium (MA) within the archetypal perovskite $MA_xCs_{1-x}PbI_3$. We find that the addition of as little as 10% caesium results in a 2.5-fold slowdown in the reorientation of the organic cation. Comparison with previous DFT and Raman studies suggests that distortion of the octahedral cavity, coupled with rigidisation of the PbI_3 cage, prevents facile rotation of the MA ion. Our work also highlights the interplay between static and dynamic disorder within OHPs, which is highly relevant for future improvements to their stability and optoelectronic properties.



Generalised crystal structure of a metal-halide perovskite.



Rotational anisotropy decays of $MA_xCs_{1-x}PbI_3$ (x = 0.0—0.3). The solid points represent averages across all runs (semitranspatent points), whilst the solid lines represent a fit of this data using Eqn. 2. The shaded region denotes the standard error of the measurements.



Extracted wobbling and jump time parameters, obtained by fitting the four anisotropy decays in Fig. 2 to Eqn. 2. The black line in (B) is digitally extracted from Ghosh, D. et al. Good Vibrations: Locking of Octahedral Tilting in Mixed-Cation Iodide Perovskites for Solar Cells. ACS Energy Lett. 2017, 2 (10), 2424–2429

Equation 2:

$$R(t) = \frac{2}{5}\left[S^2 e^{-t/\tau_{jump}} + (1 - S^2) e^{t/\tau_{wob} - t/\tau_{jump}}\right]$$

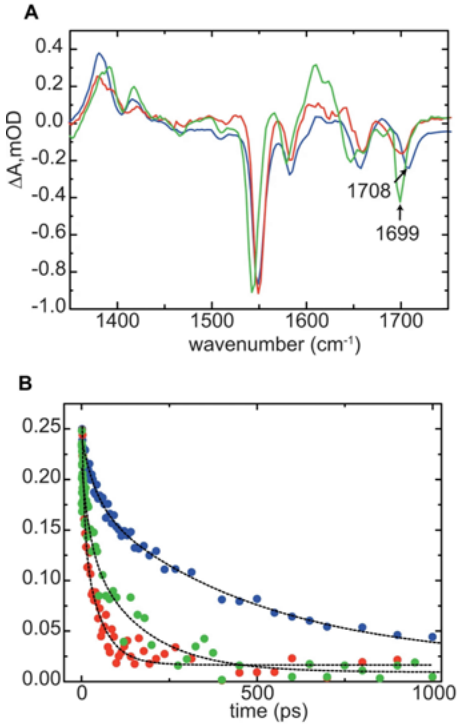
Contact: A. Bakulin (a.bakulin@imperial.ac.uk)

Functional dynamics of a single tryptophan residue in a BLUF protein revealed by fluorescence spectroscopy

K. Karadi, S.M. Kapetanaki, M. Nyitrai, A. Lukacs *(Department of Biophysics, Medical School, University of Pécs, Hungary; Szentagothai Research Center, University of Pécs, Hungary)*
K. Raics, I. Pecsi, R. Kapronczai, Z. Fekete, J. Orban *(Department of Biophysics, Medical School, University of Pécs, Hungary)*
J.N. Iuliano, J. Tolentino Collado, A.A. Gil, P.J. Tonge *(Department of Chemistry, Stony Brook University, Stony Brook, New York, USA)*
G.M. Greetham *(Central Laser Facility, STFC Rutherford Appleton Laboratory, Harwell Campus, Didcot, UK)*
M.H. Vos *(LOB, CNRS, INSERM, Ecole Polytechnique, Institut Polytechnique de Paris, France)*
S.R. Meech *(School of Chemistry, University of East Anglia, Norwich, UK)*

Blue Light Using Flavin (BLUF) domains are increasingly being adopted for use in optogenetic constructs. Despite this, much remains to be resolved on the mechanism of their activation. The advent of unnatural amino acid mutagenesis opens up a new toolbox for the study of protein structural dynamics. The tryptophan analogue, 7-aza-Trp (7AW) was incorporated in the BLUF domain of the Activation of Photopigment and PUC A (AppA) photoreceptor in order to investigate the functional dynamics of the crucial W104 residue during photoactivation of the protein. The 7-aza modification to Trp makes selective excitation possible using 310 nm excitation and 380 nm emission, separating the signals of interest from other Trp and Tyr residues. We used Förster energy transfer (FRET) between 7AW and the flavin to estimate the distance between Trp and flavin in both the light- and dark-adapted states in solution. Nanosecond fluorescence anisotropy decay and picosecond fluorescence lifetime measurements for the flavin revealed a rather dynamic picture for the tryptophan residue. In the dark adapted state, the major population of the tryptophan 104 is pointing away from the flavin and can move freely, in contrast to previous results reported in the literature. Upon blue-light excitation, the tryptophan population is reorganized, the dominant population moves closer to the flavin occupying a rigidly bound state participating in the hydrogen-bond network around the flavin molecule.

Contact: A. Lukacs (andras.lukacs@aok.pte.hu)

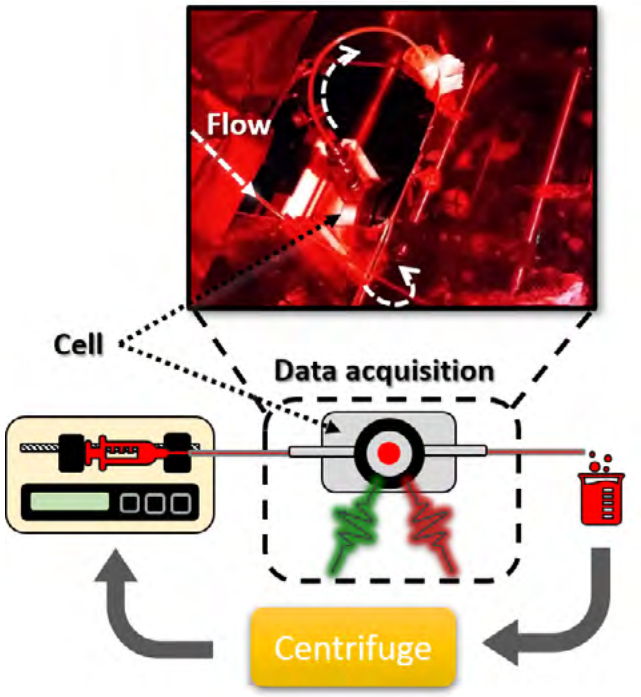


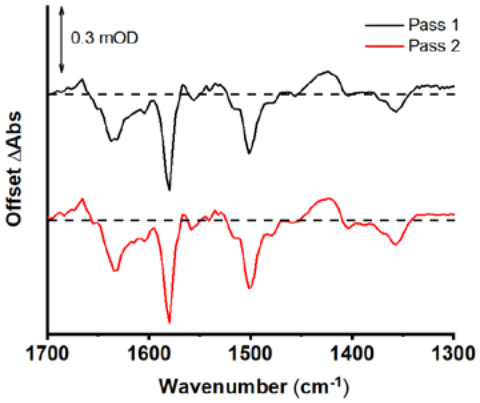
(A) TRIR spectra of dark- and light-adapted (blue and red respectively) W64F and the Y21F/Y56F/W64F AppA mutants (green) recorded at 10 ps; (B) Kinetics of the excited state of the flavin in the dark- and light-adapted (blue and red respectively) states of W64F $AppA_{BLUF}$ and in the Y21F/Y56F/W64F $AppA_{BLUF}$ (green) observed at 1380 cm^{-1}.

Overcoming the challenges of measuring the irreversible photochemical dynamics of a 'tricky' protein using time-resolved infrared spectroscopy: the B_{12}-dependent transcriptional regulator, CarH

I.S. Camacho, A.R. Jones *(Biometrology, Chemical and Biological Sciences, National Physical Laboratory, Teddington, Middlesex, UK)*
I.V. Sazanovich, E. Gozzard, M. Towrie *(Central Laser Facility, STFC Rutherford Appleton Laboratory, Harwell Campus, Didcot, UK)*
N.T. Hunt *(Department of Chemistry and York Biomedical Research Institute, University of York, UK)*

Photoreceptor proteins enable organisms to respond to their environment and can be engineered into light-activated tools and novel phototherapies. Time-resolved infrared (TRIR) spectroscopy has significant potential to elucidate the photophysical, photochemical and structural dynamics of photoreceptors. Not all such proteins are straightforwardly amenable to investigation by TRIR, however. Here we describe the development of a sample preparation and data acquisition protocol for TRIR with a 'tricky' protein: the B_{12}-dependent bacterial photoreceptor, CarH. At the concentrations required for TRIR, CarH precipitates during its irreversible photoconversion, resulting in light scattering. We therefore developed a one-way flow and sample recycling system (figure below left) that yields high quality, reproducible TRIR data (figure below right) from limited sample. This approach should be adaptable for the investigation by TRIR of similarly challenging samples, providing data that details photoreceptor dynamics and that informs the developments of light-activated, molecular biotechnologies.





Above: TRIR difference spectra at 2 ps following photoexcitation of CarH at 525 nm in aqueous buffer. The data illustrate the reproducibility of sequential acquisition 'passes' following centrifugation as shown in the figure left. Plots are offset for clarity; dashed lines indicate zero spectral amplitude; and a scale bar is indicated.

Left: Schematic of the final experimental setup (bottom) where the sample (red) flows (left to right) from a syringe pump, via tubing, through the modified cell to waste. The 'waste' is then centrifuged to remove photoconverted precipitate and the supernatant recycled for further data acquisition. Visible pump (green) and midIR probe (red) laser light are shown incident to the cell window. A photograph (top) shows the flow set up in situ under red ambient light. The direction of sample flow from the pump (out of shot) is indicated using white dashed arrows.

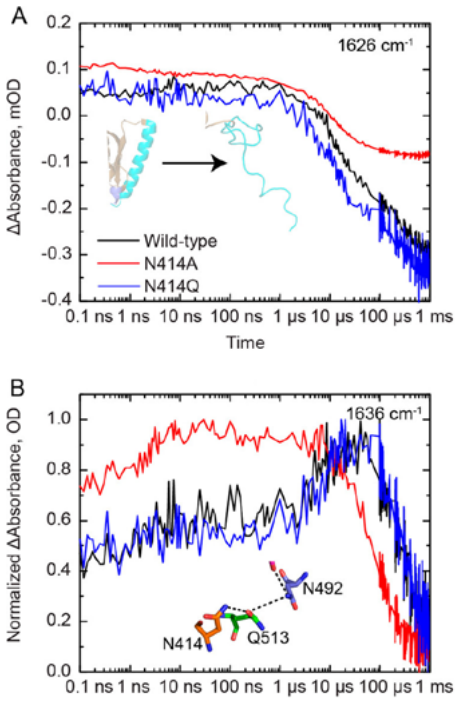
Contact: A.R. Jones (alex.jones@npl.co.uk)

Unraveling the mechanism of a LOV domain optogenetic sensor: a glutamine lever induces unfolding of the Jα Helix

J.N. Iuliano, J.T. Collado, S-Y. Shin, H.A. Woroniecka, T. Daryaee, C.L.Simmerling, P.J. Tonge *(Department of Chemistry, Stony Brook University, New York, USA)*
A.A. Gil, P.T. Ravindran, J.E. Toettcher *(Department of Molecular Biology, Princeton University, Princeton, New Jersey 08544, United States)*
A. Lukacs *(School of Chemistry, University of East Anglia, Norwich, UK; Department of Biophysics, Medical School, University of Pecs, Hungary)*
K. Adamczyk, C.R. Hall, S.R. Meech *(School of Chemistry, University of East Anglia, Norwich, UK)*
J.M. Aramini *(Structural Biology Initiative, CUNY Advanced Science Research Center, New York, USA)*
U.R. Edupuganti *(Structural Biology Initiative, CUNY Advanced Science Research Center, New York, USA; PhD Program in Biochemistry, CUNY Graduate Center, New York, USA)*
G.M. Greetham, I.V. Sazanovich, I.P. Clark *(Central Laser Facility, Research Complex at Harwell, STFC Rutherford Appleton Laboratory, Harwell Campus, Didcot, UK)*
J.B. French *(Department of Chemistry, Stony Brook University, New York, USA; Hormel Institute, University of Minnesota, USA)*
K.H. Gardner *(Structural Biology Initiative, CUNY Advanced Science Research Center, New York, USA; PhD Programs in Biochemistry, Biology, and Chemistry, CUNY Graduate Center, New York, USA; Department of Chemistry and Biochemistry, City College of New York, USA)*

Light-activated protein domains provide a convenient, modular, and genetically encodable sensor for optogenetics and optobiology. Although these domains have now been deployed in numerous systems, the precise mechanism of photoactivation and the accompanying structural dynamics that modulate output domain activity remain to be fully elucidated. In the C-terminal light–oxygen–voltage (LOV) domain of plant phototropins (LOV2), blue light activation leads to formation of an adduct between a conserved Cys residue and the embedded FMN chromophore, rotation of a conserved Gln (Q513), and unfolding of a helix (Jα-helix) which is coupled to the output domain. In the present work, we focus on the allosteric pathways leading to Jα helix unfolding in Avena sativa LOV2 (AsLOV2) using an interdisciplinary approach involving molecular dynamics simulations extending to 7 μs, time-resolved infrared spectroscopy, solution NMR spectroscopy, and in-cell optogenetic experiments. In the dark state, the side chain of N414 is hydrogen bonded to the backbone N–H of Q513. The simulations predict a lever-like motion of Q513 after Cys adduct formation resulting in a loss of the interaction between the side chain of N414 and the backbone C=O of Q513, and formation of a transient hydrogen bond between the Q513 and N414 side chains. The central role of N414 in signal transduction was evaluated by site-directed mutagenesis supporting a direct link between Jα helix unfolding dynamics and the cellular function of the Zdk2-AsLOV2 optogenetic construct. Through this multifaceted approach, we show that Q513 and N414 are critical mediators of protein structural dynamics, linking the ultrafast (sub-ps) excitation of the FMN chromophore to the microsecond conformational changes that result in photoreceptor activation and biological function.

Contact: P.J. Tonge (peter.tonge@stonybrook.edu)



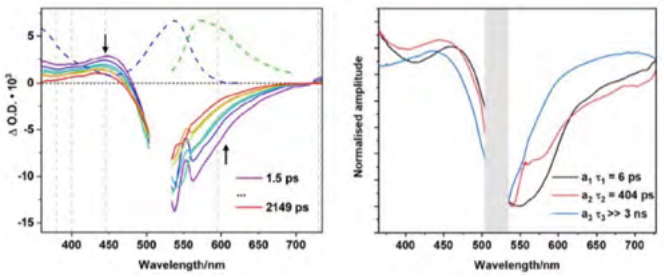
Selected kinetic traces from TRIR spectra of wild-type and mutant AsLOV2. (A) The unfolding of the Jα helix is tracked by the increase in bleach intensity at 1626 cm^{-1} for the wild-type (black), N414A (red), and N414Q (blue) AsLOV2 proteins. (B) A rise and decay of the signal at 1636 cm^{-1} is assigned to structural dynamics associated with a transient hydrogen bond between N414 and Q513 due to the rotation of Q513. This signal decays to zero with the time constant of the fourth EAS.

A time-resolved spectroscopic study of BODIPY copolymers and their application as photosensitisers for hydrogen evolution

A.A. Cullen, K. Heintz, L. O'Reilly, C. Long, M.T. Pryce *(School of Chemical Sciences, Dublin City University, Ireland)*
A. Heise, R. Murphy *(Department of Chemistry, Royal College of Surgeons in Ireland, Dublin, Ireland)*
J. Karlsson, E. Gibson *(Energy Materials Laboratory, Chemistry, School of Natural and Environmental Science, Newcastle University, UK)*
G.M. Greetham, M. Towrie *(Central Laser Facility, Research Complex at Harwell, STFC Rutherford Appleton Laboratory, Harwell Campus, Didcot, UK)*

A novel BODIPY copolymer with diethynylbenzene linkers has been studied as a photosensitising unit for photocatalytic hydrogen generation. Time-resolved infrared and transient absorption studies spanning the ps- to ns-timescales were used to probe the excited state dynamics of this photosensitising centre. Visible light irradiation of solutions containing the BODIPY copolymer together with a cobaloxime catalyst under various conditions generates hydrogen.

Reproduced from Cullen, Aoibhín A et al. A Time-Resolved Spectroscopic Investigation of a Novel BODIPY Copolymer and Its Potential Use as a Photosensitiser for Hydrogen Evolution. Frontiers in chemistry vol. 8 584060. 19 Oct. 2020, under the terms of the Creative Commons Attribution License (CC BY). doi:10.3389/fchem.2020.584060



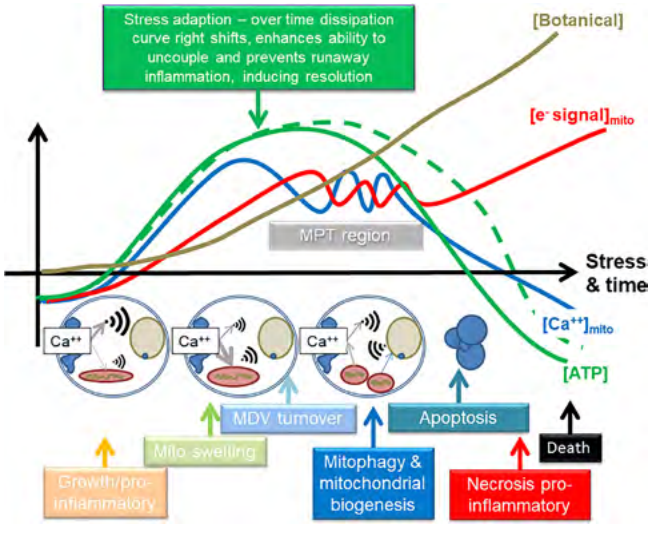
Transient absorption spectra of the BODIPY copolymer in CD_3CN following excitation at 525 nm at various time points together with the Decay Associated Spectra.

Contact: M.T. Pryce (mary.pryce@dcu.ie)

From sunscreens to medicines: Can a dissipation hypothesis explain the beneficial aspects of many plant compounds?

A.V.W. Nunn, J.D. Bell *(Research Centre for Optimal Health, Department of Life Sciences, University of Westminster, London, UK)*
G.W. Guy *(GW pharmaceuticals, Salisbury, Wiltshire, UK)*
S.W. Botchway *(Central Laser Facility, Research Complex at Harwell, STFC Rutherford Appleton Laboratory, Harwell Campus, Didcot, UK; Department of Biological and Medical Sciences, Oxford Brookes University, UK)*

Medicine has utilised plant-based treatments for millennia, but precisely how they work is unclear. One approach is to use a thermodynamic viewpoint that life arose by dissipating geothermal and/or solar potential. Hence, the ability to dissipate energy to maintain homeostasis is a fundamental principle in all life, which can be viewed as an accretion system where layers of complexity have built upon core abiotic molecules. Many of these compounds are chromophoric and are now involved in multiple pathways. Plants have further evolved a plethora of chromophoric compounds that can not only act as sunscreens and redox modifiers, but also have now become integrated into a generalised stress adaptive system. This could be an extension of the dissipative process. In animals, many of these compounds are hormetic, modulating mitochondria and calcium signalling. They can also display anti-pathogen effects. They could therefore modulate bioenergetics across all life due to the conserved electron transport chain and proton gradient. In this review paper, we focus on well-described medicinal compounds, such as salicylic acid and cannabidiol and suggest, at least in animals, their activity reflects their evolved function in plants in relation to stress adaptation, which itself evolved to maintain dissipative homeostasis.



Reproduced from A.V.W. Nunn, G.W. Guy, S.W. Botchway, J.D. Bell. From sunscreens to medicines: Can a dissipation hypothesis explain the beneficial aspects of many plant compounds? Phytotherapy Research 2020, 34(8), 1868-1888, published by John Wiley & Sons Ltd, under the terms of the Creative Commons Attribution License. doi: 10.1002/ptr.6654

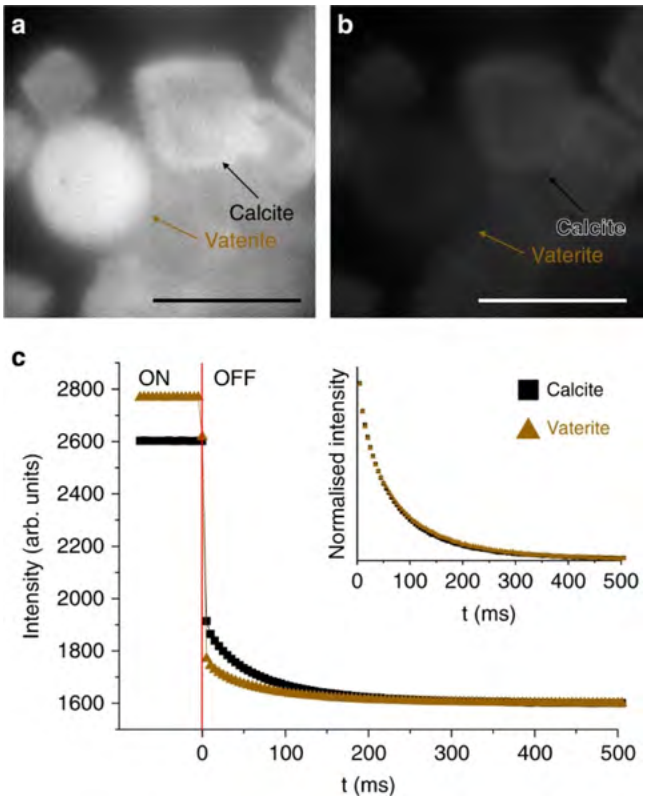
Contact: A.V.W. Nunn (a.nunn@westminster.ac.uk)

Biphasic effects of plant compounds on mitochondrial function, from a calcium-centric viewpoint
Key:
MPT = mitochondrial permeability transition
MDV = mitochondrially derived vesicles
Blue line = mitochondrial calcium concentration
Red line = electron leak leading to ROS
Green line = dissipation & ATP
Brown line = possible plant compound concentration

Controlling the fluorescence and room-temperature phosphorescence behaviour of carbon nanodots with inorganic crystalline nanocomposites

D.C. Green, S. Zhang, F.C. Meldrum *(School of Chemistry, University of Leeds, UK)*
M.A. Holden *(School of Chemistry, University of Leeds, UK; School of Physics and Astronomy, University of Leeds, UK)*
B.R.G. Johnson, J.Gala de Pablo *(School of Physics and Astronomy, University of Leeds, UK)*
M.A. Levenstein *(School of Chemistry, University of Leeds, UK; School of Mechanical Engineering, University of Leeds, UK)*
A. Ward, S.W. Botchway *(Central Laser Facility, Research Complex at Harwell, STFC Rutherford Appleton Laboratory, Harwell Campus, Didcot, UK)*

There is a significant drive to identify alternative materials that exhibit room temperature phosphorescence for technologies including bio-imaging, photodynamic therapy and organic light-emitting diodes. Ideally, these materials should be non-toxic and cheap, and it will be possible to control their photoluminescent properties. This was achieved here by embedding carbon nanodots within crystalline particles of alkaline earth carbonates, sulphates and oxalates. The resultant nanocomposites are luminescent and exhibit a bright, sub-second lifetime afterglow. Importantly, the excited state lifetimes, and steady-state and afterglow colours can all be systematically controlled by varying the cations and anions in the host inorganic phase, due to the influence of the cation size and material density on emissive and non-emissive electronic transitions. This simple strategy provides a flexible route for generating materials with specific, phosphorescent properties and is an exciting alternative to approaches relying on the synthesis of custom-made luminescent organic molecules.



Dependence of room temperature phosphorescence activation on the crystal phase. Time-resolved phosphorescence microscopy images with UV excitation (a) on and (b) 5 ms after the removal of UV excitation, and (c) intensity vs time plots for calcite (black square) and vaterite (brown triangle)-based RTP nanocomposites. The plot in (c) shows the intensity with UV light on and off, and the point at which the light is removed is shown with a vertical red line at t = 0. The normalised decay curves for calcite and vaterite overlap, indicating identical phosphorescent lifetimes (inset; scale bar 50 μm)

Contact: D.C. Green (D.C.Green@leeds.ac.uk),
F.C. Meldrum (F.Meldrum@leeds.ac.uk)

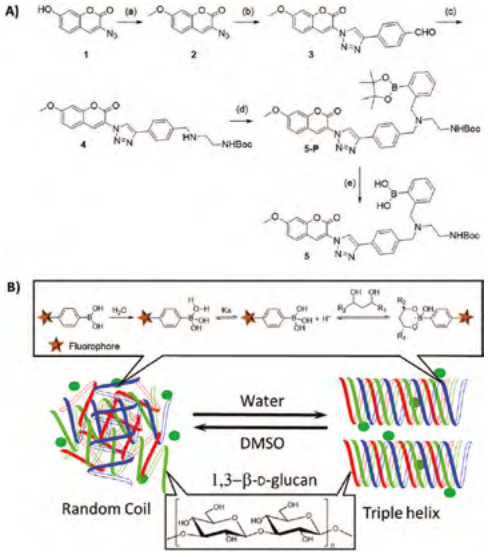
Multiphoton fluorescence lifetime imaging microscopy (FLIM) and super-resolution fluorescence imaging with a supramolecular biopolymer for the controlled tagging of polysaccharides

H. Ge, F. Cortezon-Tamarit, H-C. Wang, R.L. Arrowsmith, V. Mirabello, T.D. James, S.I. Pascu *(Department of Chemistry, University of Bath, UK)*
A.C. Sedgwick *(Department of Chemistry, University of Texas at Austin, USA)*
S.W. Botchway *(Central Laser Facility, Research Complex at Harwell, STFC Rutherford Appleton Laboratory, Harwell Campus, Didcot, UK*

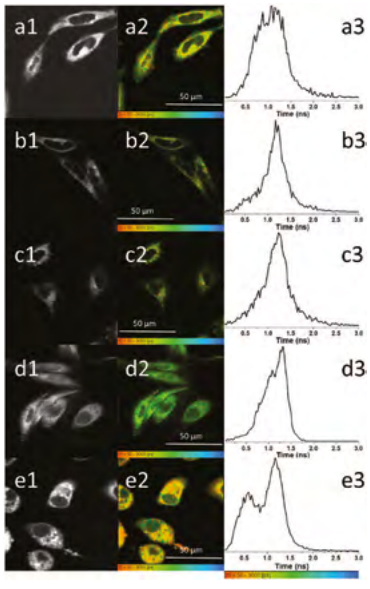
A fluorophore based on a new boronic acid derivative of coumarin and its corresponding β-D-glucan supramolecular conjugate were prepared and investigated by optical fluorescence techniques (one-photon and two-photon fluorescence spectroscopy together with time correlated singlephoton counting), AFM and Raman. The binding of this functional boronic acid to β-D-glucan from barley produced a kinetically stable nano-dimensional, soft material composite, denoted 5@β-D-glucan, with a binding affinity allowing for the retention of the complexed state even upon cellular uptake over a range of concentrations. Solution phase investigations by fluorescence spectroscopies both in single photon and two-photon excitation modes combined with *in vitro* studies (in HeLa and PC-3 cells, macrophage). The supramolecular conjugate was also evaluated as cellular imaging probes in different cancer cell lines (HeLa and PC-3) as well as J774.2 macrophages using single- and two-photon fluorescence lifetime imaging microscopy (FLIM) showing that the conjugates enter cells successfully; studies by multiphoton fluorescence confocal imaging and super-resolution imaging indicate that both 5-P and 5@β-D-glucan can enter cells and distribute in the cytoplasm at both 37°C and 4°C. Fluorescence lifetime studies confirmed for the first time the successful binding of a coumarin boronic acid with β-D-glucan which remains intact as a kinetically stable complex in aqueous environments. We describe a new coumarin-appended boronate ester as a fluorogenic reagent, which binds to both mono and polysaccharides. This new glucan hybrid could serve as cellular imaging probe: a simple coumarin boronic acid can thus act to bridge the gap between the development and application of small molecules and bio-nanomaterials for the labelling of living cancer cells. We believe that biopolymers such as β-D-glucan from barley used as a scaffold for boronate-tagging methodologies could lead to new synthetic approaches to deliver non-invasive and affordable early diagnostics for cancers which are difficult to access non-invasively such as prostate cancers.

Reproduced from Ge H, Cortezon-Tamarit F, Wang H-C, et al. Multiphoton fluorescence lifetime imaging microscopy (FLIM) and super-resolution fluorescence imaging with a supramolecular biopolymer for the controlled tagging of polysaccharides. Nanoscale, 2019,11, 9498, © 2019 The Royal Society of Chemistry, under the terms of a Creative Commons Attribution NonCommerical 3.0 Unported License. DOI: 10.1039/c8nr10344e

Contact: S.I. Pascu (s.pascu@bath.ac.uk)



Scheme 1: A) Synthesis of the pinacol-protected coumarin monoboronate ester of 5-P: (a) MeI, K_2CO_3, acetone, reflux. (100%) (b) 4-ethynylbenzaldehyde 2, $CuSO_4{\cdot}5H_2O$ (5 mol%), NaAsc (10 mol%), H_2O/EtOH = 1 : 1, rt. (80%) (c) (i) mono Boc-diamine (1 eq.), DCM/MeOH, rt. (ii) $NaBH_3CN$, MeOH, rt. (74%) (d) 2-(bromomethyl) phenylboronic acid pinacol ester, K_2CO_3, ACN, reflux. (B) Schematic representation of the β-D-glucan triple helix in water and the diagram of boronic acid fluorophore reversible binding with the diols of the 1,3-β-D-glucan. Pinacol deprotection of 5-P proceeded to 5 in situ in presence of glucan diols, at pH 8–9.5 to form the desired diol-boronate and covalently-bonded supramolecular biopolymer complex, denoted 5@β-D-glucan.

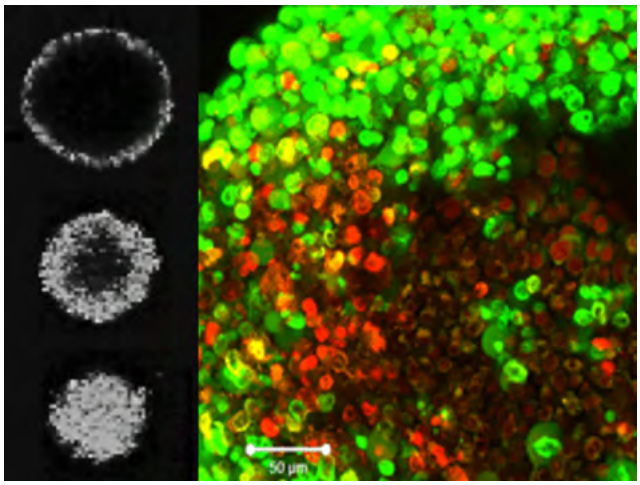


Two-photon fluorescence imaging including lifetime microscopy mapping of 5-P (100 μM in 0.5 : 99.5% DMSO : RPMI) (a_{1-2}; b_{1-2}; d_{1-2}) and 5@β-D-glucan (0.5 : 99.5% DMSO : RPMI) (c_{1-2}; e_{1-2}) in HeLa (a_{1-2}; b_{1-2}; c_{1-2}) and PC-3 cells (d_{1-2}; e_{1-2}) after 15 min incubation. Corresponding fluorescence lifetime distribution curves: a_3, b_3, c_3, d_3, e_3; λ_{ex} = 810 nm. Scale bar: 50μm. The micrographs show that 5-P and 5@β-D-glucan are capable of penetrating the cell membrane at either 4°C (b_{1-2}; c_{1-2}) or at 37°C (a_{1-2}; d_{1-2}; e_{1-2}), although the lower temperature reduces the extent of cellular uptake. Legend colours provide a direct correlation between the lifetime maps a2–e2 and the histograms showing the corresponding lifetimes distribution (a3–e3).

A dinuclear Ruthenium(II) complex excited by Near-infrared light through two-photon absorption induces phototoxicity deep within hypoxic regions of melanoma cancer spheroids

A. Raza, S. MacNeil, J.W. Haycock *(Materials Science & Engineering, University of Sheffield, UK)*
S.A. Archer, S.D. Fairbanks, K.L. Smitten, J.A. Thomas *(Department of Chemistry, University of Sheffield, UK)*
S.W. Botchway *(Central Laser Facility, Research Complex at Harwell, STFC Rutherford Appleton Laboratory, Harwell Campus, Didcot, UK)*

The dinuclear photo-oxidizing Ru^{II} complex $[\{Ru(TAP_2)\}_2(tpphz)]^{4+}$ (TAP = 1,4,5,8- tetraazaphenanthrene, tpphz = tetrapyrido- [3,2-*a*:2′,3′-*c*:3″,2″-*h*:2‴,3‴-*j*]phenazine), 1^{4+}, is readily taken up by live cells localizing in mitochondria and nuclei. In this study, the two-photon absorption cross section of 1^{4+} is quantified and its use as a two-photon absorbing phototherapeutic is reported. It was confirmed that the complex is readily photoexcited using near-infrared, NIR, and light through two-photon absorption, TPA. In 2-D cell cultures, irradiation with NIR light at low power results in precisely focused phototoxicity effects in which human melanoma cells were killed after 5 min of light exposure. Similar experiments were then carried out in human cancer spheroids that provide a realistic tumor model for the development of therapeutics and phototherapeutics. Using the characteristic emission of the complex as a probe, its uptake into 280 µm spheroids was investigated and confirmed that the spheroid takes up the complex. Notably TPA excitation results in more intense luminescence being observed throughout the depth of the spheroids, although emission intensity still drops off toward the necrotic core. As 1^{4+} can directly photo-oxidize DNA without the mediation of singlet oxygen or other reactive oxygen species, phototoxicity within the deeper, hypoxic layers of the spheroids was also investigated. To quantify the penetration of these phototoxic effects, 1^{4+} was photoexcited through TPA at a power of 60 mW, which was progressively focused in 10 µm steps throughout the entire z-axis of individual spheroids. These experiments revealed that, in irradiated spheroids treated with 1^{4+}, acute and rapid photoinduced cell death was observed throughout their depth, including the hypoxic region.



Contact: J.A. Thomas (james.thomas@sheffield.ac.uk)
S. MacNeil (s.macneil@sheffield.ac.uk)
J.W. Haycock (j.w.haycock@sheffield.ac.uk)

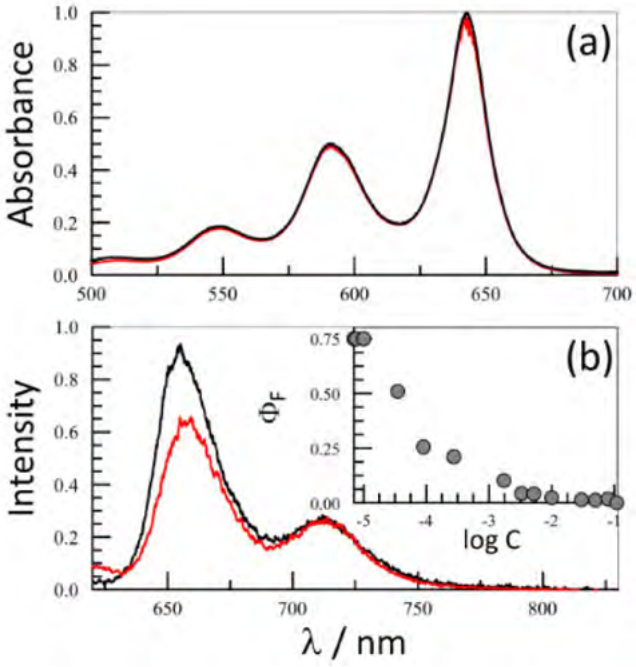
The photophysical properties of triisopropylsilyl-ethynylpentacene – a molecule with an unusually large singlet-triplet energy gap – in solution and solid phases

F.A. Schaberle, C. Serpa. L.G. Arnaut *(CQC, Department of Chemistry, University of Coimbra, Portugal)*
A.D. Ward *(Central Laser Facility, Research Complex at Harwell, STFC Rutherford Appleton Laboratory, Harwell Campus, Didcot, UK)*
J.K. Karlsson, A. Harriman *(Molecular Photonics Laboratory, SNES, Newcastle University, UK)*
A. Atahan *(Molecular Photonics Laboratory, SNES, Newcastle University, UK; Department of Polymer Engineering, Faculty of Technology, Duzce University, Turkey)*

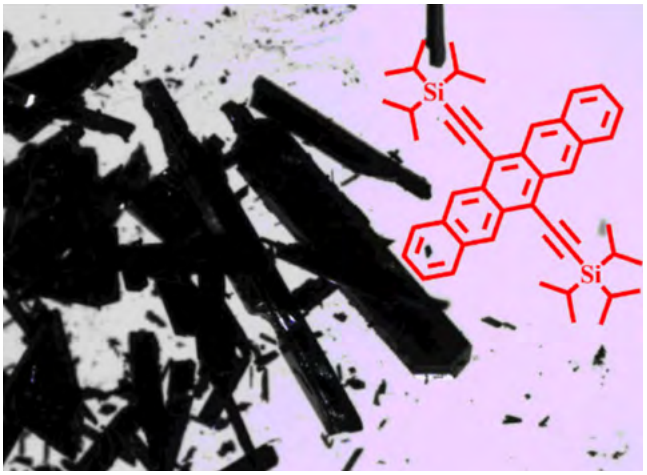
The process of singlet-exciton fission (SEF) has attracted much attention of late. One of the most popular SEF compounds is TIPS-pentacene (TIPS P, where TIPS = triisopropylsilylethynyl) but, despite its extensive use as both a reference and building block, its photophysical properties are not so well established. In particular, the triplet state excitation energy remains uncertain. Here, we report quantitative data and spectral characterization for excited-singlet and -triplet states in dilute solution. The triplet energy is determined to be 7940 ± 1200 cm^{-1} on the basis of sensitization studies using time-resolved photoacoustic calorimetry. The triplet quantum yield at the limit of low concentration and low laser intensity is only ca. 1%. Self-quenching occurs at high solute concentration where the fluorescence yield and lifetime decrease markedly relative to dilute solution but we were unable to detect excimer emission by steady-state spectroscopy. Short-lived fluorescence, free from excimer emission or phosphorescence, occurs for crystals of TIPS P, most likely from amorphous domains.

Reprinted from F.A. Schaberle. C. Serpa, L.G. Arnaut et al. Chemistry, 2(2), 545-564 (2020), © 2020 by MDPI (http://www.mdpi.org), under the terms of the Creative Commons CC BY License. DOI: 10.3390/chemistry2020033

Contact: A. Harriman (anthony.harriman@ncl.ac.uk)



(a) Comparison of absorption spectra recorded for TIPS-P in toluene at concentrations of 1 μM (black curve) and 0.05 M (red curve). The spectra, which have been normalized at the respective maxima, were recorded with a variable path length optical cell to maintain a constant absorbance at any given wavelength. (b). Examples of fluorescence spectra recorded for the above solutions, with excitation at 595 nm and the same colour scheme. Note the minor distortion of the spectrum at high concentration caused by uncompensated self-absorption. The inset shows the effect of TIPS-P concentration on the fluorescence quantum yield.

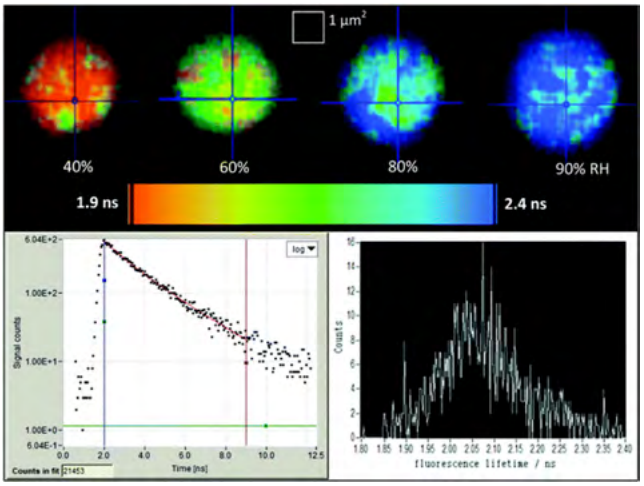


Chemical formula for TIPS-P superimposed over a photograph of crystals of the same material.

Measurement of the fluorescence lifetime of GFP in high refractive index levitated droplets using FLIM

N.M. Davidson, F.D. Pope *(School of Geography, Earth and Environmental Sciences, University of Birmingham, UK)*
P.J. Gallimore, M. Kalberer *(Department of Chemistry, University of Cambridge, UK)*
B.C. Bateman, S.W. Botchway, A.D. Ward *(Central Laser Facility, Research Complex at Harwell, STFC Rutherford Appleton Laboratory, Harwell Campus, Didcot, UK)*
M.K. Kuimova *(Department of Chemistry, Imperial College London, UK)*

Green fluorescent protein (GFP) is a widely used fluorescent probe in the life sciences and biosciences due to its high quantum yield and extinction coefficient, and its ability to bind to biological systems of interest. This study measures the fluorescence lifetime of GFP in sucrose/water solutions of known molarity in order to determine the refractive index dependent lifetime of GFP. A range of refractive indices from 1.43–1.53 were probed by levitating micron sized droplets composed of water/sucrose/ GFP in an optical trap under well-constrained conditions of relative humidity. This setup allows for the first reported measurements of the fluorescence lifetime of GFP at refractive indices greater than 1.46. The results obtained at refractive indices less than 1.46 show good agreement with previous studies. Further experiments that trapped droplets of deionised water containing GFP allowed the hygroscopic properties of GFP to be measured. GFP is found to be mildly hygroscopic by mass, but the high ratio of molecular masses of GFP to water (ca. 1500 : 1) signifies that water uptake is large on a per-mole basis. Hygroscopic properties are verified using brightfield microscope imaging, of GFP droplets at low and high relative humidity, by measuring the humidity dependent droplet size. In addition, this experiment allowed the refractive index of pure GFP to be estimated for the first time (1.72 ± 0.07). This work provides reference data for future experiments involving GFP, especially for those conducted in high refractive index media. The work also demonstrates that GFP can be used as a probe for aerosol studies, which require determination of the refractive index of the aerosol of any shape.



Top: Example fluorescence lifetime images of the same droplet of GFP in sucrose/water at 40, 60, 80 & 90% RH collected by SPCimage. Bottom left: Log time-resolved fluorescence decay of the 40% RH droplet in Tri2. Bottom right: Lifetime histogram of the same droplet, with lifetime on the x-axis and number of counts on the y-axis.

Reprinted from N.M. Davidson, P.J. Gallimore, B. Bateman, et al. Phys. Chem. Chem. Phys. 22, 14704 (2020), published by the PCCP Owner Societies, under the terms of the Creative Commons Attribution 3.0 Unported Licence. DOI: 10.1039/C9CP06395A

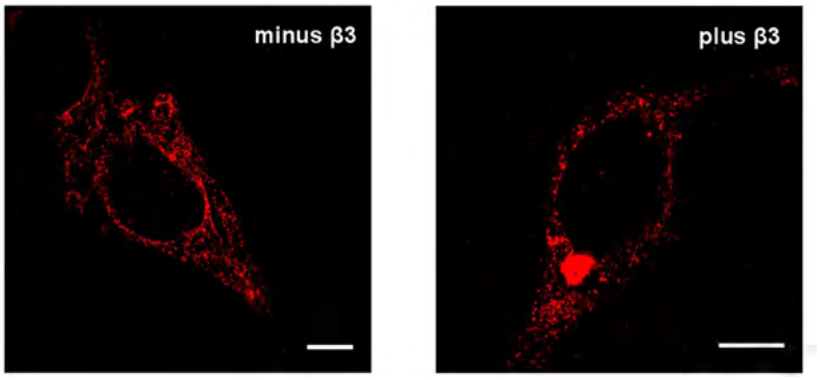
Contact: F.D. Pope (f.pope@bham.ac.uk)

Supramolecular clustering of the cardiac sodium channel Nav1.5 in HEK293F cells, with and without the auxiliary β3-subunit

S.C. Salvage, J.S. Rees, J.R. Irons, A.P. Jackson *(Department of Biochemistry, University of Cambridge, UK)*
A. McStea, M. Hirsch, L. Wang, C.J. Tynan, M.L. Martin-Fernandez *(Central Laser Facility, Research Complex at Harwell, STFC Rutherford Appleton Laboratory, Harwell Campus, Didcot, UK)*
M.W. Reed *(Department of Nuclear Physics, Research School of Physics and Engineering, Australian National University, Canberra, Australia)*
R. Butler *(Wellcome Trust/Cancer Research UK Gurdon Institute, University of Cambridge, UK)*
A.J. Thompson *(Department of Pharmacology, University of Cambridge, UK)*
C.L-H. Huang *(Department of Biochemistry, University of Cambridge, UK; Department of Physiology, Development and Neuroscience, University of Cambridge, UK)*

Voltage-gated sodium channels comprise an ion-selective ∝-subunit and one or more associated β-subunits. The β3-subunit (encoded by the SCN3B gene) is an important physiological regulator of the heart-specific sodium channel, Nav1.5. We have previously shown that when expressed alone in HEK293F cells, the full-length β3-subunit forms trimers in the plasma membrane. We extend this result with biochemical assays and use the proximity ligation assay (PLA) to identify oligomeric β3-subunits, not just at the plasma membrane, but throughout the secretory pathway. We then investigate the corresponding clustering properties of the ∝-subunit and the effects upon these of the β3-subunits. The oligomeric status of the Nav1.5 ∝-subunit in vivo, with or without the β3-subunit, has not been previously investigated. Using super-resolution fluorescence imaging, we show that under conditions typically used in electrophysiological studies, the Nav1.5 ∝-subunit assembles on the plasma membrane of HEK293F cells into spatially localized clusters rather than individual and randomly dispersed molecules.

Quantitative analysis indicates that the β3-subunit is not required for this clustering but β3 does significantly change the distribution of cluster sizes and nearest-neighbour distances between Nav1.5 ∝-subunits. However, when assayed by PLA, the β3-subunit increases the number of PLA-positive signals generated by anti-(Nav1.5 ∝-subunit) antibodies, mainly at the plasma membrane. Since PLA can be sensitive to the orientation of proteins within a cluster, we suggest that the β3-subunit introduces a significant change in the relative alignment of individual Nav1.5 ∝-subunits, but the clustering itself depends on other factors. We also show that these structural and higher-order changes induced by the β3-subunit do not alter the degree of electrophysiological gating cooperativity between Nav1.5 ∝-subunits. Our data provide new insights into the role of the β3-subunit and the supramolecular organization of sodium channels, in an important model cell system that is widely used to study Nav channel behaviour.



Super-resolution STORM imaging and of Nav1.5 ∝-subunits with and without β3-subunit at the CLF Octopus facility. Representative images are reconstructed from fluorophore AF-647 blinking in HEK293F cells co-transfected with Nav1.5-HA/EGFP and Nav1.5-HA/β3-EGFP. Bars = 5 μm.

Reproduced from Salvage, Samantha C et al. Supramolecular clustering of the cardiac sodium channel Nav1.5 in HEK293F cells, with and without the auxiliary β3-subunit. FASEB journal : official publication of the Federation of American Societies for Experimental Biology vol. 34,3 (2020): 3537-3553, under the terms of the Creative Commons Attribution 4.0 International (CC BY 4.0) License. doi:10.1096/fj.201701473RR.

Contact: S.C. Salvage (ss2148@cam.ac.uk)
A.P. Jackson (apj10@cam.ac.uk)

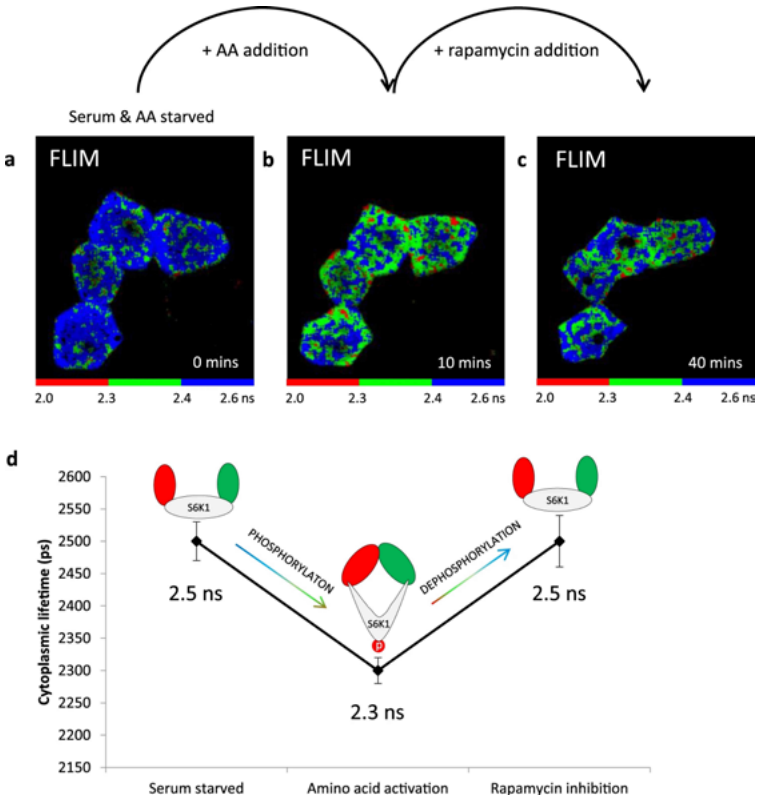
Direct imaging of the recruitment and phosphorylation of S6K1 in the mTORC1 pathway in living cells

A.R. Ahmed, C.D. Stubbs, A.W. Parker, S.W. Botchway *(Central Laser Facility, Research Complex at Harwell, STFC Rutherford Appleton Laboratory, Harwell Campus, Didcot, UK)*
R.J. Owens, R.B. Yadav *(Protein Production UK, Research Complex at Harwell, STFC Rutherford Appleton Laboratory, Harwell Campus, Didcot, UK; The Wellcome Centre for Human Genetics, Roosevelt Drive, Oxford, UK)*
R. Hitchman *(Evotec (UK) Ltd, Milton Park, Abingdon, UK)*
M. Dumoux *(Diamond Light Source, Harwell Campus, Didcot, UK; Protein Production UK, Research Complex at Harwell, STFC Rutherford Appleton Laboratory, Harwell Campus, Didcot, UK)*
C. Hawes *(Oxford Brookes University, Headington Campus, Oxford, UK)*

Knowledge of protein signalling pathways in the working cell is seen as a primary route to identifying and developing targeted medicines. In recent years there has been a growing awareness of the importance of the mTOR pathway, making it an attractive target for therapeutic intervention in several diseases. Within this pathway we have focused on S6 kinase 1 (S6K1), the downstream phosphorylation substrate of mTORC1, and specifically identify its juxtaposition with mTORC1. When S6K1 is co-expressed with raptor we show that S6K1 is translocated from the nucleus to the cytoplasm. By developing a novel biosensor we demonstrate in real-time, that phosphorylation and de-phosphorylation of S6K1 occurs mainly in the cytoplasm of living cells. Furthermore, we show that the scaffold protein raptor, that typically recruits mTOR substrates, is not always involved in S6K1 phosphorylation. Overall, we demonstrate how FRET-FLIM imaging technology can be used to show localisation of S6K1 phosphorylation in living cells and hence a key site of action of inhibitors targeting mTOR phosphorylation.

Reproduced from Ahmed, A.R., Owens, R.J., Stubbs, C.D. et al. Direct imaging of the recruitment and phosphorylation of S6K1 in the mTORC1 pathway in living cells. Sci Rep 9, 3408 (2019), under the terms of the Creative Commons Attribution 4.0 International (CC BY 4.0) License. doi: 10.1038/s41598-019-39410-z

Contact: S.W. Botchway (stan.botchway@stfc.ac.uk)

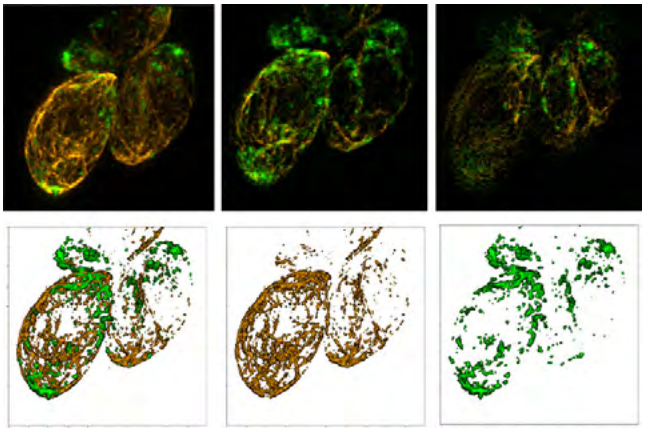


mTORC1 activation and inhibition using SensOR. (a) FLIM of serum and amino acid starved HEK293 cells expressing SensOR. (b) FLIM at 10 minutes following serine + leucine activation. (c) FLIM collected at 40 minutes after subsequent rapamycin treatment of serine + leucine activated cells for 30 minutes. (d) Summary of lifetime changes of SensOR with serum starvation, amino acid addition and rapamycin treatment from mean lifetimes and also pixel by pixel analysis of image with increased binning. Opening and closing of sensor also shown via schematics. Data representative of three independent experiments with errors representative of standard deviation, where two experiments were treated with leucine and the third treated with the combination of leucine and serine.

A highly dynamic F-actin network regulates transport and recycling of micronemes in *Toxoplasma Gondii* vacuoles

J. Periz, M. Del Rosario *(Wellcome Centre for Integrative Parasitology, Institute of Infection, Immunity & Inflammation, University of Glasgow, UK)*
A. McStea, L. Wang, M.L. Martin-Fernandez *(Central Laser Facility, Research Complex at Harwell, STFC Rutherford Appleton Laboratory, Harwell Campus, Didcot, UK)*
S. Gras *(Experimental Parasitology, Department for Veterinary Sciences, Ludwig-Maximilians-University Munich, Germany)*
M. Meissner *(Wellcome Centre for Integrative Parasitology, Institute of Infection, Immunity & Inflammation, University of Glasgow, UK; Experimental Parasitology, Department for Veterinary Sciences, Ludwig-Maximilians-University Munich, Germany)*
C. Loney *(MRC-University of Glasgow Centre for Virus Research, Sir Michael Stoker Building, Glasgow, UK)*

The obligate intracellular parasite *Toxoplasma Gondii* replicates in an unusual process, described as internal budding. Multiple daughter parasites are formed sequentially within a single mother cell, requiring replication and distribution of essential organelles such as micronemes. These organelles are thought to be formed de novo in the developing daughter cells. Using dual labelling of a microneme protein MIC2 and super-resolution microscopy, we show that micronemes are recycled from the mother to the forming daughter parasites using a highly dynamic F-actin network. While this recycling pathway is F-actin dependent, de novo synthesis of micronemes appears to be F-actin independent. The F-actin network connects individual parasites, supports long, multidirectional vesicular transport, and regulates transport, density and localisation of micronemal vesicles. The residual body acts as a storage and sorting station for these organelles. Our data describe an F-actin dependent mechanism in apicomplexans for transport and recycling of maternal organelles during intracellular development.



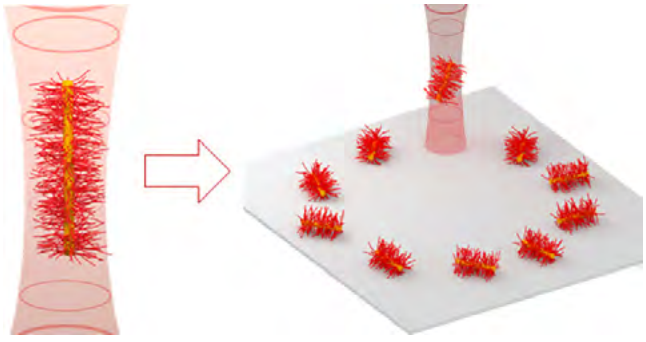
A mobile, interconnected F-actin network supports vesicular transport: 3D representative SIM image shows a continuous F-actin network connecting mother and daughter cells. Top row. 3D and z slices show continuous F-actin between mother and daughter. Bottom row. 3D surface rendered model and independent channel views comprising z stacks (z6–z15).

Contact: J. Periz (Javier.Periz@glasgow.ac.uk)
M. Meissner (Markus.Meissner@lmu.de)

Manipulation and deposition of complex, functional block copolymer nanostructures using optical tweezers

O.E.C. Gould, C.E. Boott (School of Chemistry, University of Bristol, UK)
S.J. Box, M.J. Miles *(School of Physics, University of Bristol, UK)*
A.D. Ward *(Central Laser Facility, Research Complex at Harwell, STFC Rutherford Appleton Laboratory, Harwell Campus, Didcot, UK)*
M.A. Winnik *(Department of Chemistry, University of Toronto, Canada)*
I. Manners *(School of Chemistry, University of Bristol, UK; Department of Chemistry, University of Victoria, Canada)*

Block copolymer self-assembly has enabled the creation of a range of solution-phase nanostructures with applications from optoelectronics and biomedicine to catalysis. However, to incorporate such materials into devices a method that facilitates their precise manipulation and deposition is desirable. Herein we describe how optical tweezers can be used to trap, manipulate, and pattern individual cylindrical micelles and larger hybrid micellar materials. Through the combination of TIRF imaging and optical trapping we can precisely control the three-dimensional motion of individual cylindrical block copolymer micelles in solution, enabling the creation of customizable arrays. We also demonstrate that dynamic holographic assembly enables the creation of ordered customizable arrays of complex hybrid block copolymer structures. By creating a program which automatically identifies, traps, and then deposits multiple assemblies simultaneously we have been able to dramatically speed up this normally slow process, enabling the fabrication of arrays of hybrid structures containing hundreds of assemblies in minutes rather than hours.

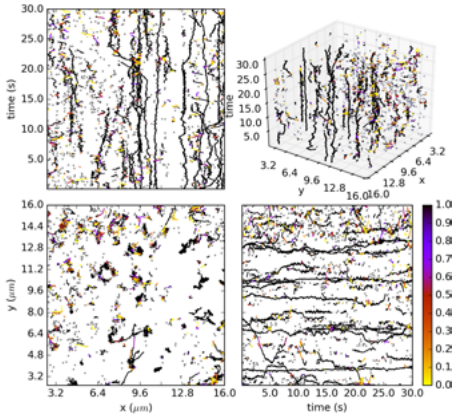


Contact M.J. Miles (mervyn.miles@bristol.ac.uk),
I. Manners (imanners@uvic.ca)

A global sampler of single particle tracking solutions for single molecule microscopy

M. Hirsch, D.J. Rolfe, L.C. Zanetti-Domingues, M.L. Martin-Fernandez *(Central Laser Facility, Research Complex at Harwell, STFC Rutherford Appleton Laboratory, Harwell Campus, Didcot, UK)*
R. Wareham, S.S. Singh *(Department of Engineering, University of Cambridge, UK)*
J.W. Yoon *(Center for Information Security Technology, Korea University, Seoul, South Korea)*
M.P. Hobson *(Department of Physics, University of Cambridge, UK)*
P.J. Parker *(School of Cancer and Pharmaceutical Sciences, King's College London, UK; Phosphorylation Laboratory, The Francis Crick Institute, London, UK)*

The dependence on model-fitting to evaluate particle trajectories makes it difficult for single particle tracking (SPT) to resolve the heterogeneous molecular motions typical of cells. We present here a global spatiotemporal sampler for SPT solutions using a Metropolis-Hastings algorithm. The sampler does not find just the most likely solution but also assesses its likelihood and presents alternative solutions. This enables the estimation of the tracking error. Furthermore the algorithm samples the parameters that govern the tracking process and therefore does not require any tweaking by the user. We demonstrate the algorithm on synthetic and single molecule data sets. Metrics for the comparison of SPT are generalised to be applied to a SPT sampler. We illustrate using the example of the diffusion coefficient how the distribution of the tracking solutions can be propagated into a distribution of derived quantities. We also discuss the major challenges that are posed by the realisation of a SPT sampler.



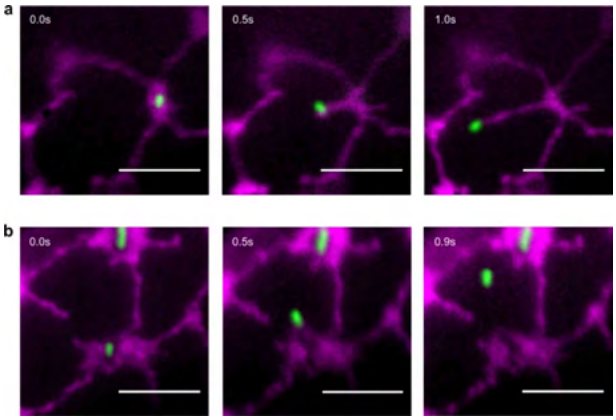
Two views on the tracking result for an SMM data set with 9761 observations. 4000 samples have been recorded. Links that appear in all samples are shown in blue. The colour of the other links indicates their frequency of occurrence as shown by the colour bar. Observations that are assigned to the clutter in all samples are shown as grey dots.

Contact: D.J. Rolfe (daniel.rolfe@stfc.ac.uk)

Miro2 tethers the ER to mitochondria to promote mitochondrial fusion in tobacco leaf epidermal cells

R.R. White, I. Leaves, J. Metz *(Biosciences, CLES, Exeter University, UK)*
C. Lin (*Department of Mathematics, Harrison Building, University of Exeter, UK; Center for Mathematical Sciences, Huazhong University of Science and Technology, Wuhan, China; Key Lab of Engineering Modeling and Scientific Computing, Huazhong University of Science and Technology, Wuhan, China)*
I.G. Castro *(Biosciences, CLES, Exeter University, UK; Department of Molecular Genetics, Weizmann Institute of Science, Rehovot 7610001, Israel)*
B.C. Bateman, S.W. Botchway, A.D. Ward *(Central Laser Facility, Research Complex at Harwell, STFC Rutherford Appleton Laboratory, Harwell Campus, Didcot, UK)*
P. Ashwin *(Department of Mathematics, Harrison Building, University of Exeter, UK)*
I. Sparkes *(Biosciences, CLES, Exeter University, UK ; School of Biological Sciences, University of Bristol, UK)*

Mitochondria are highly pleomorphic, undergoing rounds of fission and fusion. Mitochondria are essential for energy conversion, with fusion favouring higher energy demand. Unlike fission, the molecular components involved in mitochondrial fusion in plants are unknown. Here, we show a role for the GTPase Miro2 in mitochondria interaction with the ER and its impacts on mitochondria fusion and motility. Mutations in AtMiro2's GTPase domain indicate that the active variant results in larger, fewer mitochondria which are attached more readily to the ER when compared with the inactive variant. These results are contrary to those in metazoans where Miro predominantly controls mitochondrial motility, with additional GTPases affecting fusion. Synthetically controlling mitochondrial fusion rates could fundamentally change plant physiology by altering the energy status of the cell. Furthermore, altering tethering to the ER could have profound effects on subcellular communication through altering the exchange required for pathogen defence.



Mitochondria are physically attached to the ER. Consecutive TIRF microscopy images showing ER (magenta) either following a or separating b from a mitochondrion (mito, green) that has been optically trapped and moved using an automated routine; trapping laser power of 40 mW, moved 6 μm at 6 μms^{-1}. Scale bar indicates 5 μm, time is indicated in seconds (s). Cells were treated with latrunculin b.

Contact: I. Sparkes (i.sparkes@bristol.ac.uk)