CAI 4th ANNUAL SYMPOSIUM

ABSTRACTS

Session One:

8.50am Dr Jurgen Fripp

Computational radiology: training computers to turn images into information

Radiology uses medical imaging to help interpret, diagnose and treat diseases seen within the body. In recent years the increase in available compute power and maturing of algorithms provides the potential to extract innumerable quantitative features from medical images (including Positron Emission Tomography, Magnetic Resonance Imaging and Computed Tomography). Validated features (or biomarkers) derived from the images can indicate normal biological processes, pathogenic processes, or responses to therapeutic intervention. This talk will focus on the technical and validation pathway involved in the development of several clinically focused applications of computational radiology developed at The Australian eHealth Research Centre. In general, these applications aim to automate routine radiological tasks or extract imaging biomarkers. Examples will include the computational analysis of PET and MRI for neurodegeneration, MRI based radiation therapy and orthopaedics.

Session Two:

10.30am A/Prof Katie McMahon

Markers of Language Recovery in Stroke

Up to 40% of stroke survivors will experience some kind of language difficulty, known as aphasia. Of these, 60% will still be aphasic 12 months post-onset. Up until now, it has not been possible to predict response to treatment, or what type of treatment will give the best outcome for the patient. This talk will look at the results of a trial combining extensive cognitive and imaging batteries to examine what happens in the brain during recovery, and what features best predict the outcome.

10.50am Dr Desmond Tse

Parallel transmission in ultra-high field MR

Ultra-high field (UHF) MRI offers unprecedented opportunities in neuroscience research due to the increase in the images' signal-to-noise as well as contrast-to-noise ratios. Before the full potential of UHF MRI can be exploited, one of the major challenges associated with UHF MRI, namely RF inhomogeneity, must be overcome first. At UHF, the RF wavelength in vivo becomes comparable to, or even smaller than, the dimensions of the object being imaged. This causes constructive and destructive interference in the RF fields emitted from the transmit coil elements, ultimately leading to unwanted strong intensity and contrast variations in the acquired images.

Recent studies have shown that RF inhomogeneity can be ameliorated by using parallel transmission in conjunction with RF shimming and/or transmit sensitivity encoding. This talk will provide a brief introduction to these techniques and demonstrate a few examples of their applications in UHF neuro imaging.

11.10am Student Presentation: Liza van Eijk

Brain Masculinity and its relation with Autism Spectrum Traits

Males are about four times as likely as females to be diagnosed with autism spectrum disorder, and it has been argued that features of the disorder comprise exaggeratedly 'male type' behavior. Males and females also exhibit structural brain differences, and it has been suggested that autism may be linked to having an 'extreme male brain'. To investigate this hypothesis, using a landmark-based geometric morphometric approach, we developed a data-derived measure of individual differences in structural brain masculinity, and tested whether it is associated with autistic traits. **Full abstract see page 8**

11.25am Student Presentation: Javier Urriola

High-frequency oscillations for the localization of the epileptogenic focus

Epilepsy is a disease that predisposes patients to suffer from recurrent unprovoked seizures. It has been estimated that more than 50 million people have epilepsy worldwide, of which 90,000 live here in Queensland. The main aim of clinical treatment is to achieve seizure control. Currently, pharmacological treatment of epilepsy is effective in 70% of patients while the other third will become refractory to all forms of medical therapy. For patients who have refractory focal epilepsy, a tailored resection of a well-defined epileptogenic zone offers the opportunity to achieve seizure freedom. To identify the potential surgical target a multimodal structural and functional neuroimaging assessment are routinely used to localise the epileptogenic tissue. Despite the use of conventional neuroimaging techniques, a significant percentage will not undergo surgery because of the absence of clear localising evidence. **Full abstract see page 9**

11.40am Dr Reza Bonyadi

Deep learning algorithms and their applications to medical imaging

Deep learning algorithms have been around for over 10 years now and has been used in many sophisticated learning tasks including vision, audio, speech, and video. Perhaps the main reason for the popularity and success of these methods is their fascinating ability in forming multi-level abstractions of the data. Through this ability, they are capable of better generalisation of what they learn and they reduce the need for manual feature engineering. Segmentation, image reconstruction, diagnosis, brain-computer interface, and signal labelling are only a few medical imaging examples for which deep learning algorithms have been successful. This presentation will discuss basics of deep learning algorithms and some of their medical applications.

12.00pm Venkat Ramanujam

In vivo protein splicing as an efficient method to synthesize and characterize secreted cysteine-rich repeat proteins

Disulfide bridges in proteins are formed by the oxidation of cysteine residues. These cross-links play a key role in stabilizing the 3D-structure of disulfide rich polypeptides. The particular arrangement of the multiple disulfide bonds form distinct structural motifs such as the inhibitor cystine knot (ICK), found in many spider venom peptides. This arrangement has proven to be a very stable fold attracting much interest in bioengineering efforts. Recently, a new class of two domain disulfide rich peptides have been reported. This new class has been shown to contain an unusual tandem repeat of the inhibitor cystine knot (ICK) motif, where two ICK motifs are attached via a linker. The remarkable property of these peptides is that they elicit a pharmacological response that is different to that caused by either of the constituent ICK motifs individually (or as a mixture). The bivalency of these peptides therefore appears to be responsible for the observed activity. The low yield of natively folded peptide when expressed recombinantly in a bacterial host and the occurrence of homologous domains present further technical challenges for structural studies by NMR.

Here, these two-domain disulfide rich peptides are considered founding members of a broader class of bivalent peptides that are named secreted cysteine-rich repeat proteins (SCREPs). To further investigate this emerging class of peptides a general method to recombinantly produce SCREPs was developed by expressing the individual domains of SCREPs as exteins in modified intein systems, this was followed by in vivo protein ligation. This approach was tested on DkTx, the first reported SCREP, and showed a 10-fold increase in yield compared to when the peptide is expressed as a single gene. Further, segmental isotope labelling was demonstrated by selectively labeling one of the domains of DkTx. Finally, the detailed structural characterization by multidimensional NMR spectroscopy.

Lunch Session:

12.50pm Dr Kieran O'Brien

MAGNETOM PRISMA – Pushing the boundaries of Speed

The MAGNETOM PRISMA, installed at the CAI, UQ in Jan'17, is the PowerPack for exploration into advanced clinical MR research. This presentation will provide a brief overview of the benefits of upgrading the Magnetom TRIO to the MAGNETOM PRISMA with CAI, UQ's own data. We will discuss the potential impact of the maturation of Simultaneous Multi-slice (SMS) and Compressed sensing from the research to the clinical arena through examples where SMS has resulted in doing things just a little bit differently. The presentation will also provide an insight into areas of active research and development within Siemens Healthineers that aim to push the boundaries of speed beyond what is currently feasible on clinical scanners.

Session Three:

1.10pm Prof Gottfried Otting

NMR and EPR spectroscopy with chemical tags

Crystallography and, more recently, cryo-electron microscopy deliver 3D structures of proteins at atomic resolution, but provide incomplete information about the range of conformations that proteins assume in solution. NMR spectroscopy is a classical method to study protein conformations in solution, but is difficult to apply to large systems. Chemical tags offer new opportunities to assess protein structure and explore conformational space in solution. (i) Lanthanide-binding tags generate pseudocontact shifts (PCS) that can be detected in sensitive NMR experiments [1] and report on conformational variability in proteins [2]. Counterintuitively, paramagnetic lanthanides can sometimes also slow down nuclear relaxation [3]. (ii) EPR experiments allow determination of not only distances, but distance distributions between Gd^{3+} ions, which can be site-specifically attached to proteins by suitable gadolinium tags. New double-arm tags minimise the contributions from tag motions [4]. (iii) The *tert*-butyl group and the trimethylsilyl group can be site-specifically incorporated into proteins using cysteine residues or unnatural amino acids. These groups yield intense signals in the 1D ^{1}H-NMR spectrum even of proteins of high molecular weight and can be used to detect site-specific NOEs in large systems without isotope labelling [5,6].

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[1] Nitsche, C. and Otting, G. (2017) Pseudocontact shifts in biomolecular NMR using paramagnetic metal tags. *Prog. NMR Spectr.* **98-99**, 20-49.

[2] Pearce, B.J.G., Jabar, S., Loh, C.-T., Szabo, M., Graham, B. and Otting, G. (2017) Structure restraints from heteronuclear pseudocontact shifts generated by lanthanide tags at two different sites. *J. Biomol. NMR* **68**, 19-32.

[3] Orton, H.W., Kuprov, I., Loh, C.-T. and Otting, G. (2016) Using paramagnetism to slow down nuclear relaxation in protein NMR. *J. Phys. Chem. Lett.* **7**, 4815-4818.

[4] Welegedara, A.P., Yang, Y., Lee, M.D., Swarbrick, J.D., Huber, T., Graham, B., Goldfarb, D. and Otting, G. (2017) Double-arm lanthanide tags deliver narrow Gd^{3+}–Gd^{3+} distance distributions in DEER measurements. *Chem. Eur. J.*, in press.

[5] Chen, W.-N.; Kuppan, K. V.; Lee, M. D.; Jaudzems, K.; Huber, T.; Otting, G. *J. Am. Chem. Soc*. 137, 4581-4586 (2015).

[6] Jabar, S., Adams, L., Wang, Y., Aurelio, L., Graham, B. and Otting, G. (2017) Chemical tagging with t-butyl and trimethylsilyl groups for measuring intermolecular NOEs in a large protein-ligand complex. *Chem. Eur. J.*, in press.

2.00pm Dr Yas Tesiram

Spectroscopic Imaging - new and emerging methods

Abstract TBA

2.20pm Dr Zach Houston

Nanoparticles and the blood-brain barrier: a multimodal toolkit for the development of nano-theranostics for the treatment of glioblastoma

Nanomaterials come in a plethora of designs, shapes, chemical formulations, sizes, and ionic forms and can be readily tuned to surpass many of the biological barriers within the body, but have had limited success in surpassing the final frontier of barriers: the blood-brain barrier (BBB). While their size is a significant advantage of nanoparticles for their delivery to solid tumour masses, it is also their Achilles' heel for crossing the BBB. A major area of interest for nanoparticle therapeutics is the delivery to glioblastoma (GBM), as it is the most aggressive form of brain cancer. Herein we report the use of simultaneous PET-MRI to develop a toolkit for monitoring tumour progression and its effect on BBB integrity of a spontaneous transgenic glioma model[1,2], for the purpose of establishing when the BBB is compromised enough for nanoparticles to cross. A series of T1, T2, and dynamic contrast enhanced MRI images along with simultaneous PET of 18FDOPA were used to devise a set of in vivo imaging markers that could establish tumour volume and a measure of BBB integrity. PET was again used to analyse the ability of a 64Cu labelled bispecific antibody targeted hyperbranched polymer (HBP)[3] cross the BBB at different stages of GBM progression. As expected, larger tumour volumes and a higher degree of leaky vasculature correlate with increased BBB permeability by the HBP. This measure can be applied in the future to different sized nanoparticles and other materials to enable better development of BBB-penetrating nanocarriers.

References

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[2] Chow, L.; Endersby, R.; Zhu, X.; Rankin, S.; Qu, C.; Zhang, J.; Broniscer, A.; Ellison, D.; Baker, S. Cancer Cell 2011, 19, 305-316.

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2.40pm Student Presentation: Theo Crawford

In vivo optical imaging of B. anthracis protective antigen targeting endogenous mouse squamous cell carcinoma

Tumour Endothelial Marker 8 (TEM8) is an endothelial transmembrane protein associated with cell migration and adhesion[1]. TEM8 overexpression has been identified in colorectal carcinoma[2], melanoma[3], breast cancer[4], non-small cell lung cancer[5], Lewis lung carcinoma[6], and osteosarcoma[7] but is undetectable in normal healthy tissues. Thus the tumour specific overexpression of TEM8 could be used as a potential imaging biomarker for diagnostic or therapeutic purpose. A candidate for targeting TEM8 is the naturally occurring ligand Protective Antigen (PA), a protein produced by *B. anthracis* which translocates toxins[8]. Utilizing PA's biological machinery has potential for targeted therapeutics[9], but there is a lack of *in vivo* imaging data to support the tumour specific localization. We have recombinantly produced PA and labelled it with sulfo-Cyanine5 dye (PA-Cy5) to identify tumour specific localization using optical imaging. **Full abstract see page 10**

Session Four:

3.45pm A/Prof Jeff Harmer

Developing A Structural Model of a P450-Ferredoxin Complex from Orientation-Selective Double Electron-Electron Resonance Spectroscopy

Class I cytochrome P450 enzymes (CYPs) receive two electrons sequentially from NAD(P)H via a cognate ferredoxin reductase and ferredoxin, and are responsible for tasks as diverse as mitochondrial vitamin D3 hydroxylation and bacterial antibiotic synthesis. The interaction of these Class I CYPs with their cognate ferredoxin is specific and the first electron-transfer step is rate limiting. In order to reconstitute the activity of diverse CYPs, structural characterization of CYP:ferredoxin complexes is necessary, but little structural information is available. We report a rigid-body model of such a complex (CYP199A2:HaPux) in frozen solution derived from distance and orientation restraints gathered by orientation-dependent double electron-electron resonance (DEER), an EPR technique.

References

[1] Bowen, A. M., et al. Orientation-Selective DEER Using Rigid Spin Labels, Cofactors, Metals, and Clusters. In Structural Information from Spin-Labels and Intrinsic Paramagnetic Centres in the Biosciences. Editors: C. R. Timmel and J. R. Harmer. **2014**, vol 152**:** 283-327.

[2] Abdalla, J. A. B., et al. "Characterisation of the paramagnetic 2Fe-2S (+) centre in palustrisredoxin-B (PuxB) from Rhodopseudomonas palustris CGA009: g-matrix determination and spin coupling analysis." Physical Chemistry Chemical Physics **2012**, 14(18): 6526-6537.

4.05pm Damion Stimson

Development of novel lipophilic [^{18}F]thiosemicarbazone metal fluoride complexes

There is a need to develop more lipophilic metal-fluoride moieties which can be incorporated into new radiopharmaceuticals for potential applications in brain imaging with PET. This is because fluorine-18 labelling strategies previously reported have resulted in the formation of hydrophilic metal-[^{18}F]fluorine complexes that are unsuitable.

In this work, we have synthesised a range of substituted thiosemicarbazone metal chloride complexes. We have investigated the exchange of the chloride with a variety of ligands and have been able to synthesise the methoxide and nitrate analogues. Complexes of thiosemicarbazone gallium fluoride were prepared by reacting the nitrate derivative with fluoride. [^{18}F]diphenylthiosemicarbazone gallium fluoride was prepared in a formulation of saline with ethanol and used for preliminary PET imaging studies in mice highlighting the potential of these compounds for applications in neuroimaging.

4.25 Student Presentation: Joshua Harbort

Orientation-Selective DEER: a tool for molecular distance and orientation characterization in haem proteins

Dipole electron-electron resonance spectroscopy (DEER) is a technique that can obtain distance and orientation information in molecular systems that are challenging for other modalities, such as in protein complexes. DEER is typically performed on systems that have had pairs of paramagnetic spin labels covalently attached to member proteins, which introduces a set of complications[i]. Wild-type proteins require mutation to provide appropriate labelling sites, spin label flexibility introduces rotamer distributions to measurements, and typical spin labels have relatively isotropic unpaired electrons, so that orientation information cannot be determined with a single experiment.

Many proteins incorporate intrinsic paramagnetic centres with well-defined positions, however, that can ameliorate these difficulties. Metal-centred electrons are also often highly anisotropic, which allows orientation information to be measured without multiple mutants. **Full abstract see page 11**

4.40pm Student Presentation: Anna Gemmell

Dual-stimuli-responsive polymeric micelles for monitoring therapeutic efficacy in vivo

The development of ‘smart’ nanomedicines capable of visualising stimuli-responsive delivery of therapeutics continues to provide a promising outlook for the future treatment of cancer. Advances in in vivo molecular imaging technology, such as MRI, PET and optical imaging, have increased our ability to successfully visualise and monitor the delivery and effect of nanomedicines in the body. However, understanding the fate of these nanomaterials in delivering therapeutic agents homogenously across a heterogeneous tumour mass in vivo remains largely unknown. **Full abstract see page 12**

Brain Masculinity and its relation with Autism Spectrum Traits

Liza van Eijk[1], Lachlan T. Strike[2], Katie L. McMahon[3], Paul M. Thompson[4], Greig I. de Zubicaray[5], Margaret J. Wright[2,3], Brendan P. Zietsch[1]

[1]School of Psychology, University of Queensland,[2]Queensland Brain Institute, University of Queensland, [3]Centre for Advanced Imaging, University of Queensland, [4]Keck School of Medicine, University of Southern California, Los Angeles, [5]Institute of Health and Biomedical Innovation, Queensland University of Technology

Males are about four times as likely as females to be diagnosed with autism spectrum disorder[1], and it has been argued that features of the disorder comprise exaggeratedly 'male type' behavior[2]. Males and females also exhibit structural brain differences, and it has been suggested that autism may be linked to having an 'extreme male brain' [2,3]. To investigate this hypothesis, using a landmark-based geometric morphometric approach[5], we developed a data-derived measure of individual differences in structural brain masculinity, and tested whether it is associated with autistic traits.

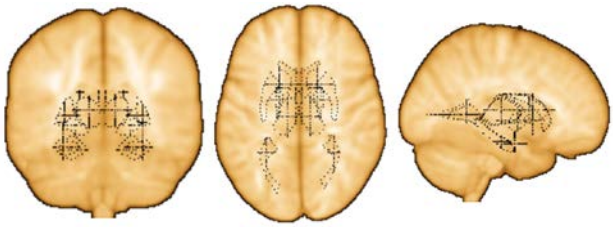

Figure 1. 934 Landmarks placed on native brain.

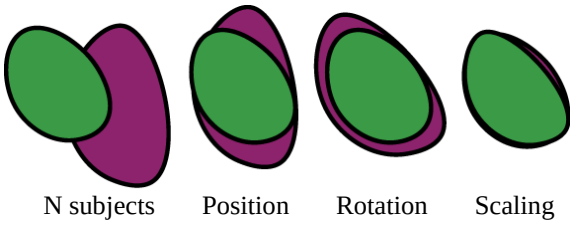

Figure 2. Generalized Procrustes Analysis.

Method: Using the Queensland Twin IMaging (QTIM) sample we identified 934 landmarks on subcortical structures and, with SPM automatically transferred the landmarks from the standard template to native space (Fig. 1). A Generalized Procrustes Analysis[6] was applied to the landmark coordinates, removing variation in brain size, position, orientation and rotation (Fig. 2). A Principal Component Analysis then yielded uncorrelated brain shape variables, which were used to predict sex in a Linear Discriminant Analysis (LDA). The resulting linear combination of the shape variables, reflecting the dimension that best discriminates between the sexes, yielded a score for each individual which reflects the masculinity (degree of maleness) of their brain. We then predicted sex in the Human Connectome Project (HCP) sample based on the LDA function. The partial correlation between the brain masculinity scores and autistic traits, controlling for sex, age, and /or general cognitive ability, was tested in a sub-sample from QTIM.

QTIM: N = 1040 (22.42 ± 3.33 years; 65% female), N = 40 test-retest scans, N = 249 Autism Spectrum Quotient
4 Tesla (Siemens Bruker), T1/TR/TE = 700/1500/3.35 ms; flip angle = 8°, slice thickness = 0.9 mm, voxel size = 0.9× 0.9 × 0.9 mm^3

HCP: N = 1113 (28.80 ± 3.70 years; 54% female)
3 Tesla (Siemens Connectome Skyra), T1/TR/TE = 1000/2400/2.14 ms; flip angle = 8°, slice thickness = 0.7 mm, voxel size = 0.7 x 0.7 x 0.7 mm^3

Results: Using the QTIM scans to create the linear combination to discriminate between the sexes resulted in an accuracy of 75.65% of predicting sex in the independent HCP sample (p <.001). Deriving the score in the HCP sample and validating in QTIM showed an accuracy rate of 75.10% (p <.001). Test retest results showed a very good reliability of the brain masculinity scores (r =.955, p <.001, n =40). After controlling for sex, age and acquisition, brain masculinity did not correlate with autistic traits in the total sample (r =.037, t =1.02, ns ,n =249); however, in males we found a correlation in line with our predictions (r =.180, t= 2.29, p =.023, n=80), while there was no significant effect in females (r = -.084, t=-1.55, ns, n=169).

Conclusion: We developed and validated a data-derived measure of structural brain masculinity. In males, we found that brain masculinity was associated with autistic traits in a normal population.

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High-frequency oscillations for the localization of the epileptogenic focus

Javier Urriola[1, 2], *Lars Marstaller*[1 2], *Steffen Bollman*[1], *Hana Burianova*[1 2], *David Reutens*[1]

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Epilepsy is a disease that predisposes patients to suffer from recurrent unprovoked seizures. It has been estimated that more than 50 million people have epilepsy worldwide, of which 90,000 live here in Queensland. The main aim of clinical treatment is to achieve seizure control. Currently, pharmacological treatment of epilepsy is effective in 70% of patients while the other third will become refractory to all forms of medical therapy. For patients who have refractory focal epilepsy, a tailored resection of a well-defined epileptogenic zone offers the opportunity to achieve seizure freedom. To identify the potential surgical target a multimodal structural and functional neuroimaging assessment are routinely used to localise the epileptogenic tissue. Despite the use of conventional neuroimaging techniques, a significant percentage will not undergo surgery because of the absence of clear localising evidence.

Recently, high-frequency oscillations (HFOs), which are brief, low-amplitude discharges on the electroencephalogram (EEG), have attracted much interest, not only because they are increased within the seizure onset zone, but also because the resection of the tissue that generates HFOs is associated with seizure-free outcomes[1].

Our aim was to investigate the neural substrate of epileptic spikes associated to HFOs (red spikes) using simultaneous EEG and functional magnetic resonance imaging (EEG-fMRI) of refractory patients undergoing presurgical investigations. We hypothesized that red spikes can be used in EEG-fMRI to reveal regions of epileptogenic tissue. To achieve this, we used a topographic voltage-map matching approach[1] on 10 patients with focal epilepsy, using data obtained during clinical EEG monitoring and simultaneous EEG-fMRI acquisition to reveal significant haemodynamic changes associated with red spikes. To validate our results, we compared the fMRI maps with current clinical neuroimaging techniques (fig.1), showing that EEG-fMRI of red spikes can provide lateralising and localising information of the epileptogenic tissue. The non-invasiveness of this approach could improve the presurgical evaluation and help the planning of invasive EEG or respective surgery.

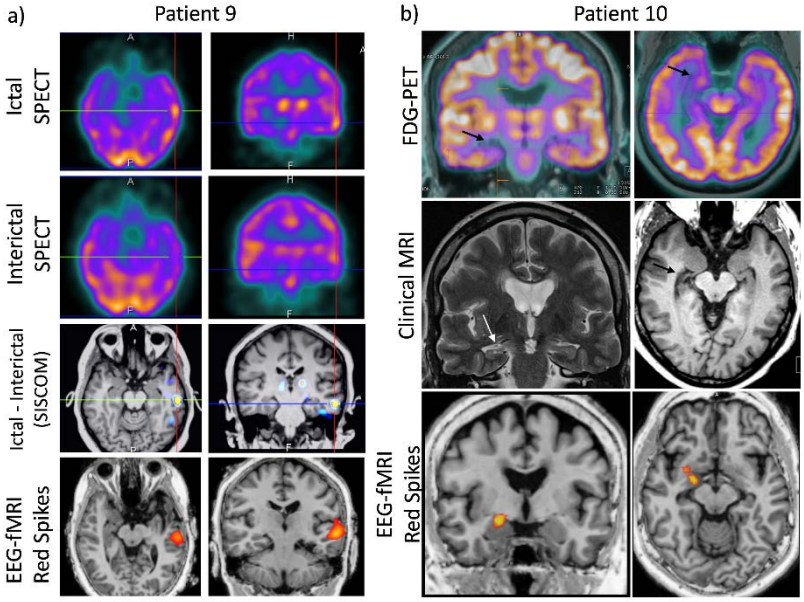

Fig 1- Concordant results between clinical investigations and EEG-fMRI of red spikes. A) Patient with focal epilepsy showing a region of pathological substrate on lateral aspect of the left temporal on ictal and interictal SPECT and on EEG-fMRI. B) Patient with right hippocampal sclerosis (epileptogenic lesion) showed as hypo-perfused region on FDG-PET, loss of volume and architecture on structural MRI and concordant BOLD changes on EEG-fMRI.

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In vivo optical imaging of B. anthracis protective antigen targeting endogenous mouse squamous cell carcinoma

Theo Crawford[1], *Margaret Veitch*[2], *Nicholas Fletcher*[1], *Ian Brereton*[1], *James W. Wells*[2], *Mehdi Mobli*[1], *Yasvir Tesiram*[1]

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Tumour Endothelial Marker 8 (TEM8) is an endothelial transmembrane protein associated with cell migration and adhesion[1]. TEM8 overexpression has been identified in colorectal carcinoma[2], melanoma[3], breast cancer[4], non-small cell lung cancer[5], Lewis lung carcinoma[6], and osteosarcoma[7] but is undetectable in normal healthy tissues. Thus the tumour specific overexpression of TEM8 could be used as a potential imaging biomarker for diagnostic or therapeutic purpose. A candidate for targeting TEM8 is the naturally occurring ligand Protective Antigen (PA), a protein produced by *B. anthracis* which translocates toxins[8]. Utilizing PA's biological machinery has potential for targeted therapeutics[9], but there is a lack of *in vivo* imaging data to support the tumour specific localization. We have recombinantly produced PA and labelled it with sulfo-Cyanine5 dye (PA-Cy5) to identify tumour specific localization using optical imaging. *In vivo* studies were performed using an HPV38E6E7.FVB mouse model which produces squamous cell carcinoma in response to repeated dosing with UVB irradiation. Rapid tumour uptake was observed, reaching a maximum relative fluorescent intensity (RFI) within 8 hours (**Fig. 1**). Assuming the tumour RFI decays exponentially with time, the tumour half-life of PA-Cy5 was calculated to be ~12 hours. The background RFI from the fur and skin reached baseline levels 24 hours post injection. These results suggest that PA is an excellent imaging tool as its rapid uptake and low background is ideal for multiple imaging techniques. *Ex vivo* analysis showed significantly higher signal in the tumour versus the blood, heart, spleen, lungs, kidneys, liver and skin ($P < 0.002$). Mean RFI of the tumours was ~3 times higher than the skin and liver, and ~4 times higher than the kidneys 48 hours after injection. Using PA-Cy5 we show the first *in vivo* images of tumour specific localization. The tumour uptake is highly efficient, allowing exclusive detection 24 hours after injection. The rapid tumour uptake and low background highlights the potential of PA as a tumour specific imaging agent. PA would likely translate to an extremely useful PET imaging agent that has high specificity to multiple tumours and low uptake in clearance organs.

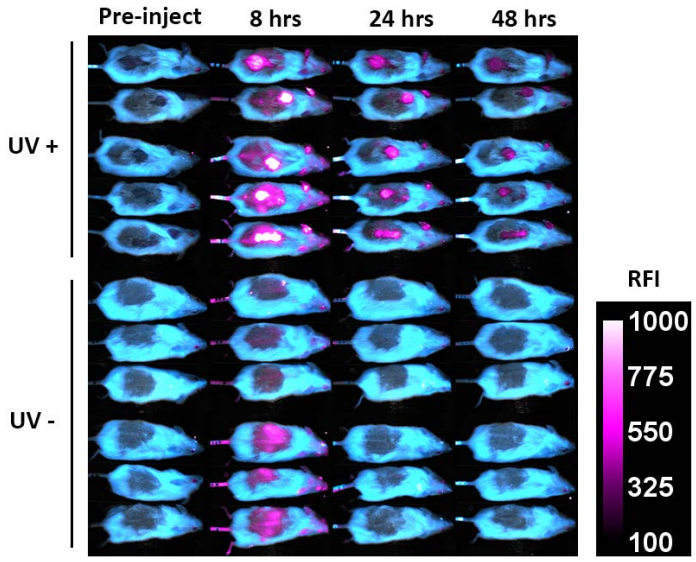

Figure 1. Spectrally unmixed *in vivo* optical images of HPV38E6E7.FVB mice injected with PA-Cy5. Two groups of mice were imaged prior to injection, 8, 24, and 48 hours post injection. The UV+ group (n=5) was exposed to UV irradiation 3 times a week for ~20 weeks to produce squamous cell carcinomas on their back. The UV- group (n=6) is of a similar age to the UV+ group but have not been exposed to UV irradiation. A series of images was taken at each time point with multiple excitation wavelengths, measuring the emission at 700 nm. Optical spectra were modelled for fur auto-fluorescence and PA-Cy5 fluorescence. These models were applied to each of the images to isolate the fur (cyan) and PA-Cy5 (magenta). The calibration bar represents the relative fluorescence intensity (RFI) of PA-Cy5.

[1] K. Hotchkiss *Exp. Cell Res.* **2005**, *305*, 133 [2] B. St Croix *Science* **2000**, *289*, 1197 [3] M. Cullen *Cancer Res.* **2009**, *69*, 6021 [4] M. Opoku-Darko *Cancer Invest.* **2011**, *29*, 676 [5] F. Kuo *Mol. Pharm.* **2014**, *11*, 3996 [6] R. Mehran *Cancer Res.* **2014**, *74*, 2731 [7] C. Cao *Sci. Rep.* **2016**, *6*, 23419 [8] A. Liu *Trends Microbiol.* **2014**, *22*, 317 [9] A. Rabideau *Sci. Rep.* **2015**, *5*, 11944

Orientation-Selective DEER: a tool for molecular distance and orientation characterization in haem proteins

Joshua Harbort[1], *Jeanette Stok*[2], *James D Voss*[2], *Jeffrey Harmer*[1, 2],

[1] EPR Group, Centre for Advanced Imaging, The University of Queensland
[2] School of Chemistry and Molecular Biosciences, The University of Queensland

Dipole electron-electron resonance spectroscopy (DEER) is a technique that can obtain distance and orientation information in molecular systems that are challenging for other modalities, such as in protein complexes. DEER is typically performed on systems that have had pairs of paramagnetic spin labels covalently attached to member proteins, which introduces a set of complications[i]. Wild-type proteins require mutation to provide appropriate labelling sites, spin label flexibility introduces rotamer distributions to measurements, and typical spin labels have relatively isotropic unpaired electrons, so that orientation information cannot be determined with a single experiment. Many proteins incorporate intrinsic paramagnetic centres with well-defined positions, however, that can ameliorate these difficulties. Metal-centred electrons are also often highly anisotropic, which allows orientation information to be measured without multiple mutants.

Haem-centred proteins are particularly challenging because of limitations in the range of molecular orientations that can be accessed to fully characterise the orientation behaviour of the system, and recent attempts have made no attempt to analyse the anisotropy of the system[ii,iii]. In this work, an unprecedented range of molecular orientations has been sampled (**Fig 1**) and combined with distance information measured with an alternative, non-orientation selective technique (RIDME[iv]), and orientation-selective simulations to provide both a distance and a relative orientation estimate for the two spin centres in a spin-labelled cytochrome P450.

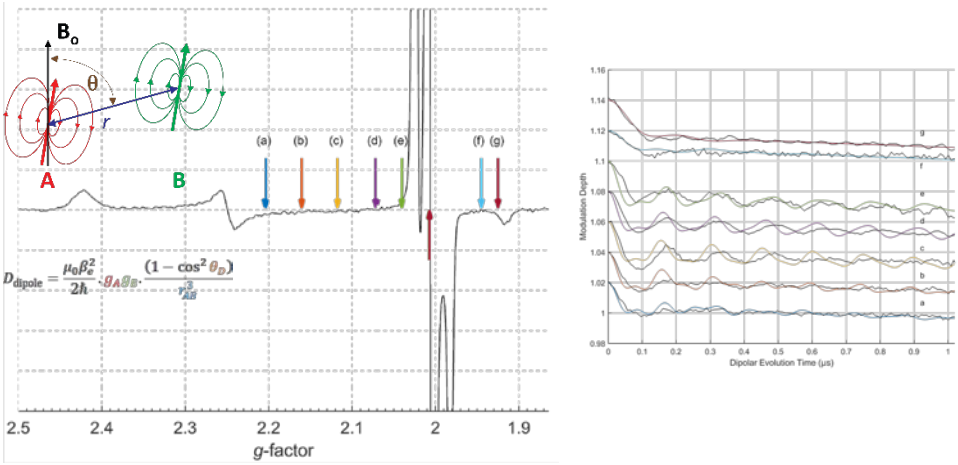

Figure 1. (Right) Orientation-selective DEER traces (colour) and simulations (black) corresponding to the pump (downwards arrows) and detection (upwards arrows, overlapping here) DEER pulse positions shown (left). The best-fitting simulations are shown here. (Inset, left) shows a diagram of the distance and orientation between the two spin centres in the DEER experiment, along with the dipole interaction equation. The angle θ corresponds to the different pump positions.

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Dual-stimuli-responsive polymeric micelles for monitoring therapeutic efficacy *in vivo*

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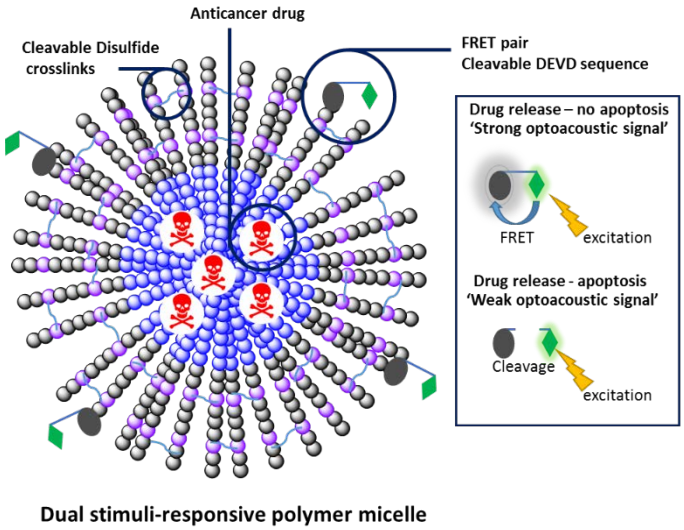
The development of 'smart' nanomedicines capable of visualising stimuli-responsive delivery of therapeutics continues to provide a promising outlook for the future treatment of cancer. Advances in *in vivo* molecular imaging technology, such as MRI, PET and optical imaging, have increased our ability to successfully visualise and monitor the delivery and effect of nanomedicines in the body. However, understanding the fate of these nanomaterials in delivering therapeutic agents homogenously across a heterogeneous tumour mass *in vivo* remains largely unknown.

Recently, an alternative *in vivo* imaging technique, optoacoustic imaging (OI), has been shown to reveal important pharmacokinetic and pharmacodynamic information about nanomedicines[1]. By combining the high contrast of optical imaging and the high resolution of ultrasound, OI is an emerging technique that surpasses the capabilities of its constituent techniques providing superior deep tissue imaging through sensitive detection of both endogenous and exogenous probes. As has been previously demonstrated[2], OI is also capable of monitoring molecular interactions through incorporation of an appropriate donor-acceptor FRET pair. Here we report on the development of novel polymeric assemblies through the synthesis of a dual stimuli-responsive amphiphilic crosslinked polymer micelle using RAFT polymerisation. This micelle was subsequently loaded with doxorubicin for cancer therapy and labelled with an optoacoustic FRET pair responsive to cell-death biomarkers, thus enabling visualisation and real-time monitoring of nanocarrier delivery, uptake and therapeutic effect in tumour tissue. Release of the drug from the micelle is enhanced through cleavage of disulfide crosslinkers, while the subsequent therapeutic effect is monitored through the evolution of an optoacoustic response as a result of FRET that arises following degradation of the nanostructure through a caspase-3-cleavable (DEVD) peptide sequence.



Scheme 1: Schematic of the dual stimuli-responsive polymer micelle comprising disulfide crosslinks for stimuli release of Dox and a FRET pair for OI to monitor therapeutic effect through cleavage of a caspase-3 responsive DEVD peptide.