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Book of Abstracts

PLENARY SESSIONS

Are we winning the fight against global disease?

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Drug discovery approaches have & continue to evolve as advancements in technology & knowhow allow; but, thus far, failed to fulfil the premise of a strong drug arsenal to fight global disease. It appears that more & more failure happen in Phase 2 and 3 clinical trials with the main culprit being efficacy. This has rendered open innovation almost impossible in large pharma as they constantly shift their trends towards life style drugs; allowing a notably quicker & greater reward on their investments. The sector has spent over $190 billion in M&A deals since January 2015 across a multitude of companies and pipeline assets; on target to meet or exceed the unprecedented $450 billion record set last year. However, the US FDA only approved 44 drugs in 2014 with 17 of them deemed first-in-class; and pricing the cost of true innovation or lack of at $26 billion per drug. Considering the purchasing power of a single US dollar across the world, this cannot continue and must invoke intervention of better collaborations and partnerships to fulfil the pipeline; also call upon conscientious policy makers & governmental agencies to facilitate this effort through public- private funding models. Failure to intervene would unequivocally start the countdown to supreme colonization by global disease.

Targeting cancer-associated genes using nanoparticle delivery of gene silencing

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Cancer is a disease of uncontrolled growth. Normal cells grow and multiply in a highly regulated and controlled manner, however, errors in the genetic blueprint of cells can mean this highly controlled regulation of growth is lost[1]. In addition, cancer cells can acquire the ability to move, spread and invade distant sites in the body. In cancer, proteins that control cell division (mitotic proteins) and cell movement (cytoskeletal proteins) are often disrupted or aberrantly expressed[2]. These proteins are important anticancer drug targets. However resistance to therapies that target mitotic and cytoskeletal proteins is a major hindrance to cure. Our laboratory has identified key cytoskeletal proteins that impact cell growth, survival and metastatic spread. Using nanoparticle delivery of short-interfering RNA (siRNA) we have shown that we can block cell growth, sensitise cancer cells to chemotherapy and reduce metastatic spread both *in vitro* and *in vivo*. Collectively, targeting mitotic and cytoskeletal proteins aberrantly expressed in cancer using nanoparticle of siRNA offers a promising approach to treat aggressive and drug resistant tumours.

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Orexin: sleep, addiction and much more.

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Orexins A and B (hypocretins 1&2) and their two receptors (OX_1R or OX_2R) were discovered in 1998 by two different groups. These neuropeptides are expressed in a few thousand cells strictly located in the lateral hypothalamus (LH), but their projections and receptor distribution throughout the brain are widespread. Remarkably, pre-pro peptide and double (OX_1R / OX_2R) receptor KOs mice reproduce a sleep phenotype known in humans as narcolepsy / cataplexy, characterized by the absence of orexin producing cells in the LH, and no or very little orexin the cerebrospinal fluid. Null mutation of the individual OX_1R or OX_2R in mice show little or only moderate sleep phenotypes. Orexin is a master regulator of the sleep-wake cycle, with high activity of the LH orexin cells during wake and almost none during sleep. Almorexant, a dual orexin receptor antagonist (DORA), was effective in inducing sleep in volunteers and insomnia patients. Although almorexant's development was stopped, no less than 4 orexin receptor antagonists have reached phase II or III in insomnia, including SB-649868, Filorexant (MK 6096) and Suvorexant (MK 4305). Suvorexant (Belsomra®, Merck) has been approved in the US and Japan in 2014. The appropriate balance of antagonism at the two receptors for sleep is a point of debate: in rodent models OX_2R antagonism alone appears sufficient to induce sleep, whereas OX_1R antagonism is largely devoid of effect. At least 2 selective OX_2R antagonists have reached phase II in insomnia. Orexin is involved in a number of other functions including reward and feeding, where OX_1R (possibly OX_2R) antagonists display antiaddictive properties in rodent models of alcohol, smoking, and drug selfadministration. The role of orexin in a number of other domains such as pain, mood, anxiety, migraine and neurodegenerative diseases is being investigated.

Traditional Medicinal Plants as a Source of New Drugs

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Medicinal plants have been used as traditional treatments for numerous human diseases for thousands of years and in many parts of the world. In rural areas of developing countries, they continue to be used as the primary source of medicine. About 80% of the people in developing countries use traditional medicines for their health care. The natural products derived from medicinal plants have proven to be an abundant source of biologically active compounds, many of which have been the basis for the development of new lead chemicals for pharmaceuticals. As there are approximately 500,000 plant species occurring worldwide, of which only 1% has been phytochemically investigated, there is great potential for discovering novel bioactive compounds. In this presentation, research performed in our laboratories on the properties of traditional medicinal plants from Australia and Borneo will be described. This work has mainly focused on applications in clinical and veterinary microbiology but also includes investigations of the anti-diabetes, anti-obesity and anti-cancer activities. The selection of plants based on ethnomedicinal knowledge, as opposed to random screening, resulted in a remarkable 'hit rate' of positive activity. In addition, many plant extracts demonstrated different modes of action compared to commercial drugs which raises the expectation that novel bioactive molecules will be identified. The application of rigorous scientific examination validates the use of medicinal plants and helps to encourage further research into the traditional and cultural knowledge associated with their use.

Lessons from a vaccine developer!

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This presentation will highlight the remarkable contribution vaccines have made in reducing global morbidity and mortality from infectious diseases. It will also confront the challenges and surprising failures of some recent vaccine development programs as well as drawing on my own experience as an inventor, entrepreneur and investor in new vaccine technologies.
This will include an assessment of challenges in bringing new vaccines to the market as well as the huge returns on investment to be made when a technology succeeds.

In the Company of Friends – How MecRx Went from an Idea to a Fully Fledged Pharma Startup

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Low molecular weight inhibitors of neuronal calcium ion channels

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Neuropathic pain is an aberrant pain state that results from nerve damage following surgery, trauma or disease. Various estimates suggest that at least 3% of the world's population is afflicted with this debilitating condition, with up to 5% of postoperative patients being affected. Symptoms often fail to respond to existing treatments and the off-label prescription of CNS- active drugs is common. Safe and effective therapies for neuropathic pain are therefore in high demand. N-Type calcium channels (Cav2.2 channels) are strongly implicated in chronic and neuropathic pain and their inhibitors have been widely pursued. This approach has been best validated by Ziconotide (Prialt®), a synthetic version of the peptide ω-conotoxin MVIIA found in the venom of a fish-hunting marine cone snail *Conus magnus*. This peptide selectively targets Cav2.2 channels and is one of the very few effective drugs used to treat intractable chronic pain. Its intrathecal mode of delivery and narrow therapeutic window, however, means that it is not an ideal treatment option.

We[1] and others have been developing small-molecule inhibitors of Cav2.2 channels as possible alternatives to Ziconotide. Our latest results in this quest will be presented.

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Rebuilding human kidneys: how do we move from organoid to medical application?

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The prevalence of chronic kidney disease is rising at 6% per annum across the globe, however treatment options for end stage renal disease have not changed in more than half a century. This represents a significant imperative for the development of novel treatments for kidney disease. Regenerative medicine is hoped to provide such alternatives. One approach involves the regeneration of kidney cell types via the directed differentiation of human pluripotent stem cells. We have developed a protocol for the differentiation of human pluripotent stem cells into kidney organoids containing collecting duct epithelium, patterned and segmenting nephrons, surrounding interstitium and vasculature. This involves the stepwise induction of posterior primitive streak, anterior and posterior intermediate mesoderm and ultimately progenitors of all epithelial and non-epithelial components of the final organ. The development of this protocol opens up the possibility of patient-derived disease modelling, drug screening, cellular therapies and even the bioengineering replacement renal tissue. In this presentation we will discuss these various applications and what progress has been made to date.

Validating of protein: ligand complexes: Reconciling theory and experiment

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Understanding how a given ligand, such as a pharmaceutical drug molecule, interacts with its receptor whether this is a therapeutic target, metabolizing enzyme or carrier molecule is central to the success of drug design and understanding the pharmacokinetic properties of molecules more generally. In particular, knowing the 3-dimensional structure of a ligand:receptor complex in detail is central to computational drug discovery. The three dimensional structure of a molecule cannot be observed directly, however. Instead, data from X-ray diffraction and/or Nuclear Magnetic Resonance (NMR) is combined with atomic interaction parameters in order to generate a structural model. In the case of high resolution X-ray diffraction data the experimental density term dominates. However, at medium to low resolution (> 1.5 Å) the quality of the final structure is primarily determined by the reliability of the molecular interaction and geometric parameters used during refinement.[1] Highly optimized and well-validated interaction parameters (force fields) are available for common biomolecules such as proteins, but not for most drug-like ligands or enzyme co-factors. This frequently leads to errors in the proposed structures, the misinterpretation of experimental data and the failure of drug discovery efforts.[2,3] The presentation will focus on what extent existing structures can be trusted and how molecular dynamics simulations in combination with free energy calculations can be used to validate and correct the structures of ligand:protein complexes.[4] In addition, how molecular simulation techniques more generally can be used to gain insight into the process associated with ligand-receptor interactions not assessable to experiment will be discussed.[5-7]

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Tripeptide motifs as a drug design paradigm: recent case studies

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Tripeptide motifs (i.e. three contiguous amino acids) are often over-represented in Nature, and they also have the correct physical size to function as efficient ligands for protein targets. We contend that if these motifs constitute a useful and minimal biological recognition signal, they may similarly provide a useful new paradigm for discovering peptides and small organic molecule mimics that are useful modulators of biological function.[1] This paper describes tripeptide motifs and their occurrence in Nature and provides three case studies where they have provided the major design rationale for antimicrobial,[2] haematopoietic[3] and anticancer[4] drug leads with novel modes of action. Chirally sensitive, self-assembling tripeptides[5] with applications in medicine will also be described briefly

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Targeted Topical Drug Delivery: New Strategies and Opportunities

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The oral route is the simplest and most convenient means to administer systemically acting therapeutics. However, drugs to treat localized diseases are also frequently administered orally when efficacy following topical delivery is compromised by poor local bioavailability. Although this can be successful, it is clearly not the most rational approach. Much larger doses must be given to the patient than would be the case with topical delivery and this increases the risk of side effects. Several methods can be used to improve the efficiency of topical drug delivery – (i) chemical modification to produce "transport-friendly" prodrugs [1], (ii) formulation techniques to increase drug thermodynamic activity and so improve local bioavailability through increased partitioning [2] and (iii) physical approaches, such as iontophoresis where the potential gradient complements the concentration gradient that governs passive diffusion across a rate-limiting membrane [1] or fractional laser ablation that compromises the membrane barrier and provides parallel diffusional pathways [3]. Here, we illustrate how these different strategies can be used to improve cutaneous, ocular and buccal delivery and show how biodistribution is a more insightful parameter than bioavailability in understanding the efficacy, or lack thereof, of topical therapies.

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Polymers and the BioNano Interface

Thomas Davis

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Raising funds for early-stage R&D

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My talk will describe options for attracting funds in early stage drug development in Australia. The talk will not address traditional grant applications. Rather it will focus on attraction of funds from investors, companies and other government sources. The talk will reference some non-confidential case studies and example – projects involving CSIRO.

Cell based measurement tools to explore therapeutic action

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CONCURRENT SESSIONS

Concurrent session 1: Mechanisms of diseases and drug development

Targeting the malaria parasite's stress response to overcome artemisinin resistance

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Artemisinin derivatives are usedin combination regimens, as first line antimalarials in most countries where malaria is endemic. However their usefulness is threatened by the emergence of drug resistance, manifesting as decreased sensitivity of the early ring blood stage. We undertook a detailed kinetic analysis of the drug responses of K13 wildtype and mutant isolates of *Plasmodium falciparum*sourced from a region in Cambodia (Pailin). We demonstrate that ART treatment induces growth retardation and an accumulation of ubiquitinated proteins, indicative of a cellular stress response that engages the ubiquitin/ proteasome system. We show that resistant parasites exhibit lower levels of ubiquitinated proteins and delayed on-set of cell death, indicating an enhanced cell stress response. We found that the stress response can be targeted by inhibiting the proteasome. Accordingly, clinically-used proteasome inhibitors strongly synergize ART activity against both sensitive and resistant parasites, including isogenic lines expressing mutant or wildtype K13. Synergy is also observed against *P. bergheiin vivo*. Ourwork provides a rationale for improving the detection of ART resistance in the field and for treatment strategies that can be employed in areas with ART resistance.

Pro-apoptotic drugs to treat infectious diseases

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We have previously shown that chronic Hepatitis B virus (HBV) infection can be eliminated in pre-clinical models by selectively killing infected hepatocytes[1,2]. Tumour necrosis factor siganlling normally promotes cellular activation and cell survival through the induction of NFkappa-B transcriptional activity. Integral to this signaling cascade are a set of molecules called inhibitor of apoptosis (IAP) proteins. If IAPs are genetically ablated or chemically inhibited, TNF no longer promotes cellular activation and instead it induces apoptosis. We harnessed the TNF that is produced locally in the milieu of infection to preferentially kill infected cells by antagonizing IAP function. Infected cells are primed to undergo TNF driven apoptosis, in the absence of IAPs, because these cells upregulate TNF receptor-1. We have expanded our initial HBV studies and translated our therapeutic intervention to several other intracellular pathogens including HIV, HTLV-1, *Mycobacterium tuberculosis* and *Burkholderia pseudomallei*. We found that TNF can also be harnessed in these infections to kill the pathogen reservoir including the latent viral pool in HIV infection.

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Nanomedicine: Targeted nanodelivery in cancer and corneal haze

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We found out that a cell permeable dominant negative survivin (SurR9-C84A) competitively inhibited endogenous survivin and blocked the cell cycle at G1/S phase. Nanoencapsulation in mucoadhesive chitosan nanoparticles (CHNP) substantially increased its bioavailability and serum stability. We investigated the anti-cancer activity of alginate coated chitosan nanoparticles (CHNPs) encapsulating SurR9-C84A with aptamers targeting EpCAM and nucleolin. We incorporated three locked nucleic acid (LNA) modifications in each sequence in order to enhance the stability of these aptamers. Confocal microscopy revealed binding of the LNA-aptamers to their specific markers with high affinity. In colon cancer mouse xenograft model, the muco-adhesive LNA decorated nanoparticles showed 6-fold higher internalization and tumour specific uptake. A higher intensity of nanobullets was observed in both periphery and core of the multicellular tumour spheroids compared to non-targeted CHNP-SurR9-C84A. The nanoparticles were found to be the highly effective as they led to a 2.26 fold ($p < 0.05$) reduction at 24 h and a 4.95 fold reduction ($p \leq 0.001$) fold reduction in the spheroid size at 72 h. The tumour regression was 4 fold higher in mice fed on nanoparticle diet when compared to control diet. The nanobullets were able to show a significantly high apoptotic ($p \leq 0.001$) and necrotic index in the tumour cell population ($p \leq 0.01$) when compared to void NPs (without SurR9-C84A). Thus, in conclusion, our nanoparticles have shown highly promising results and therefore deliver a new conduit towards the approach of cancer-targeted nanodelivery.

Alkali burn is a frequently occurring ocular injury that resembles ocular inflammation caused by eye allergies, infection, and refractive surgeries. Our results confirmed that combination of SurR9-C84A with TSA worked in synergy to heal ocular injury and inflammations due to alkali burn and led to the regeneration of ocular tissue by increasing clathrin, claudin, survivin, and TGF-β and reversal of alkali burn by suppressing IL-1α and MMP-9 without inducing haze.

Engineered bovine colostrum: an alternative to antibiotics for *Clostridium difficile* infections

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The increased incidence of antibiotic resistant “superbugs” in hospitals has amplified the use of broad spectrum antibiotics worldwide. An unintended consequence of antimicrobial treatment is the disruption of protective gut microbiota, resulting in susceptibility to opportunistic pathogens, such as Clostridium difficile. Paradoxically, treatment for C. difficile infection also involves antibiotic use, leaving patients continuously susceptible to re-infection. C. difficile is the most common cause of bacterial-induced, antibiotic-associated diarrhoea in hospitals in the developed world. Colonisation of the human colon and the establishment of fulminant disease is a complex process that depends on the ingestion of C. difficile spores, which enter the gastrointestinal tract and germinate in the intestinal mucosa. The resulting vegetative cells cause disease following the production of the major toxins TcdA and TcdB. These toxins are released by pathogenic strains of C. difficile and cause a scope of disease that ranges from mild diarrhoea to more severe conditions such as pseudomembranous colitis and toxic megacolon. This serious health threat has led to an urgent call for the development of new therapeutics to reduce or replace the use of antibiotics to treat bacterial infections. To address this need, we, together with Immuron Pty Ltd, have developed a colostrum-based immunotherapy through the immunisation of pregnant dairy cows with particular C. difficile antigens. This has resulted in a colostrum product that contains a high concentration of antigen-specific antibodies that target essential virulence components of C. difficile, specifically, spores, vegetative cells and TcdB. We have shown that administration of TcdB-specific colostrum alone, or in combination with colostrum that targets spores or vegetative cells, prevents and treats primary C. difficile disease in mice. Furthermore, this colostrum product also provides significant protection against disease relapse in mice. Collectively, these results indicate that C. difficile-specific colostrum may be used as a passive immunotherapy strategy for the prevention or treatment of primary C. difficile disease as well as disease recurrence.

Concurrent session 2: Drug delivery

Advancing antimicrobial therapy by inhalation aerosol technology

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Infections caused by multi-drug resistance (MDR) bacteriahave become a global health threat[1]. With no antibiotics appearingsoon the treatment horizon, new strategies are necessary to tackle the MDR issues. Specific strategies using bacteriophage therapy will be discussed for respiratory infections.Bacteriophages (phages)are viruses which infect and replicate inside bacteria. These phages are highlyspecific, not affecting the human microbiome, effective against MDR bacteria,and able to penetrate biofilms. Phages for respiratory administration have been mostly formulated in liquid preparations. PEV2 is a podovirus phage against *Pseudomonas aeruginosa*[2]. Wehave prepared inhalable powder formulationsof PEV2 by spray dryingand spray freeze-drying. These formulations showedexcellent*in vitro*properties: high aerosol performance, containingviable phages, andbeingphysicallystableduring long-term storage[3]. Animal studies are being conducted to study the *in vivo* efficacy of these phage formulations.

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Bringing gold-standard oxytocin therapy to all women to prevent post-partum haemorrhage

Michelle McIntosh

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Lipid-based nanomaterials for delivery of therapeutic proteins and peptides

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Biopharmaceuticals, including therapeutic proteins and peptides, represent the fastest growing class of new pharmaceuticals with application as treatments for auto-immune disorders, cancer and cardiovascular disease. Significant efforts have converged towards the design and development of more sophisticated delivery systems for protein-based pharmaceuticals, able to ensure controlled release of these bioactive compounds as well as protect the encapsulated therapeutic from denaturing processes such as enzymatic or acidic hydrolysis. Lipid-based nanomaterials are particularly useful for the encapsulation of amphiphilic proteins and peptides[1], as their bilayer structure mimics the native cell membrane environment and may assist in retaining the protein in a functionally active form. Control of drug release rates from such materials has been shown to depend on the structural parameters of the lipid mesophase. In order to advance the use of such materials we must understand the relationship between the nanostructure of the lipidic material, the encapsulated protein and their end use in drug delivery.

The research presented aims to elucidate the fundamental physicochemical interactions between encapsulated proteins and peptides and lipidic materials suitable for drug delivery. In order to screen the large compositional space associated with the design of such materials, we focus on high-throughput methodologies, and the use of large national and international synchrotron facilities such as the Australian Synchrotron, the Bragg Institute and ASTRID2 synchrotron, Denmark. The impact of encapsulated protein on the lipid nanostructure has been determined for a wide range of proteins and peptides[2-4]. In addition the effect of the lipid nanostructure on the conformation and activity of the proteins has been determined directly within the lipidic material[5]. Small-angle neutron scattering data on contrast matched lipidic materials has allowed the determination of protein location within the material for the first time[6]. The diffusion coefficient for amino acids encapsulated within a range of different cubic phase lipids, as obtained by pulsed field gradient NMR, was used to predict their release profile. Predicted release profiles tracked closely the measured release profiles, indicating that NMR determined diffusion measurements can be used to accurately predict the release profile of a model drug.

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Exploring the solid state of nanocrystallised drug in liposomes and its changes upon exposure to biorelevant media using synchrotron based SAXS

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Purpose: Commercially available liposomal formulations are mostly limited to the intravenous route of administration. A recently reported approach to nanocrystallise encapsulated drug *in situ* within liposomes[1] generated increasing interest to explore the solid state properties of these drug nanocrystals within confinement. The main advantages of these formulations are their ability to increase drug loading and modulate the dissolution rate of poorly water soluble drugs. We are interested in exploring the potential use of these particles in oral delivery applications, where the impact of the digestion of lipids and resulting drug dissolution rate are unknown. In this study the effect of bile salt is explored on the nanocrystallised ciprofloxacin liposomes in order to explore the solid state changes of the nanocrystallised ciprofloxacin liposome upon exposure to biorelevant media for the oral delivery route of administration.

Methods: Drug diffraction and crystallinity of the drug within the liposome is determined in the wider angle (higher q scattering vector) with synchrotron based small angle X-ray scattering. This method to characterize nanocrystal drug within liposomal confinement was previously done on Doxil formulations[2].

Results:

The nanocrystallised ciprofloxacin liposomes is precipitated as base hydrate inside the liposome and upon increasing bile salt concentration, this formulation showed a different drug diffraction profile indicating a polymorphic transformation on exposure to intestinal media.

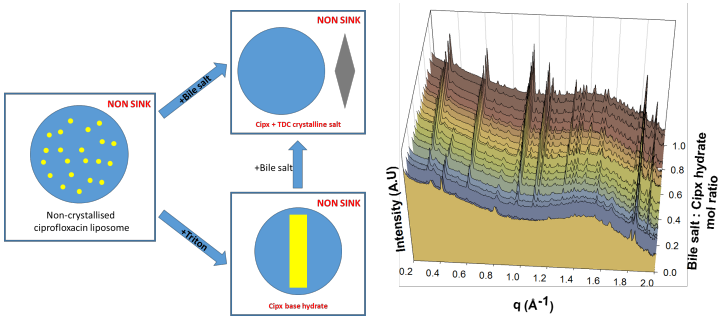

Figure: (left) schematics of the formation of ciprofloxacin base hydrate for the crystallised ciprofloxacin liposome which upon addition of bile salt would transition to the ciprofloxacin taurodeoxycholate salt crystals (right) sSAXS diffractograms of nanocrystallised ciprofloxacin liposomes in suspension and its transition to the new salt crystal upon increasing bile salt concentration

Conclusions: Digestion of nanocrystallised ciprofloxacin liposomes resulted in destruction of the liposomes, and a transformation of the drug to a new polymorphic form not previously reported. This has important implications for the dissolution rate and oral application of these materials.

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Concurrent session 3: Mechnisms of diseases and drug development

Disarming bacterial virulence: a contemporary approach to antimicrobial chemotherapy

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The increasing resistance of bacteria to antimicrobial drugs poses a serious threat to health. This problem has emerged because all current antibacterial drugs act by interfering with growth and thus apply strong selective pressure for the development of antimicrobial resistance (AMR). We have developed a novel strategy to deal with AMR, which specifically affects bacterial pathogens without retarding their growth. The targets of this treatment are the DNA-binding proteins that regulate expression of bacterial virulence. These virulence regulators sense when pathogens have reached the site of infection, after which they activate the genes required to cause disease.

In a proof-of-principle study, we identified regacin, a small molecule inhibitor of RegA. RegA is a DNA-binding protein that activates virulence in *Citrobacter rodentium,* an intestinal pathogen of mice that is used as a surrogate for infections with enteropathogenic and enterohaemorrhagic strains of *E. coli*. We have shown that regacin binds RegA within its DNA-binding domain, thus neutralising RegA's activity. Regacin was highly effective at inhibiting *C. rodentium* virulence gene expression in vitro, and completely inhibited virulence when administered perorally to mice, either 15 minutes before or up to 12 hours after infection with *C. rodentium* (1).
One of the bacterial pathogens we are currently studying is enterotoxigenic *E. coli* (ETEC), an important pathogen of humans and various domestic animals, as well as the most important cause of travellers' diarrhoea. A DNA-binding protein similar to RegA regulates virulence of most ETEC strains and is also susceptible to inhibition by small molecules. We are currently screening for inhibitors of ETEC in different classes that could ultimately be developed into drugs to prevent or treat infections with ETEC, including infantile gastroenteritis and travellers' diarrhoea.

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Understanding the molecular and cellular specificity of mineralocorticoid signalling in cardiovascular disease

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Novel high affinity inhibitors of the MYST family of chromatin regulators are effective in inducing cellular senescence

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KAT6A (MOZ), a member of the MYST lysine acetyltransferase (KAT) family, was originally discovered in recurrent translocations leading to an aggressive form of acute myeloid leukaemia. Interestingly *KAT6A* is contained within the 12th most commonly amplified genomic regions across
all cancers. KAT6A is essential for correct body patterning and organogenesis during prenatal development where it regulates the expression of key transcription factors, in particular *Hox* genes (1,2). We have shown that KAT6A is required for the formation of haematopoietic stem cells during development (3) and their maintenance in adults (4). articularly relevant for oncogenesis, we have shown that KAT6A suppresses cellular senescence (5) and is required for the progression of B cell lymphoma- driven by overexpression of the cMyc oncogene (6).
Since pathological changes to the chromatin landscape are a typical features of cancer and because of the involvement of the MYST KATs in cancer, we set out to develop small molecule inhibitors of KAT6 with hope that these will become a novel class of anticancer therapeutics targeting histone acetylation.
We have produced a series of specific inhibitors of KAT6 with single digit nM IC50. Our studies show these are reversible acetyl-coA competitors and directly inhibit histone acetylation at KAT6 target loci in cells. The inhibitors induce cellular senescence, without DNA damage, in a p16INK4A and p19ARF-dependent fashion. Senescence is accompanied by gene expression changes typical of genetic loss of KAT6A function. Furthermore we have shown that these inhibitors are effective in potentiating oncogeneinduced senescence in pre-clinical cancer models.

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Neuroprotective effect of a blood-brain barrier permeable aurone in *Caenorhabditis elegans* models.

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Small molecules able to modify the underlying causes of neurodegenerative diseases such as Alzheimer's are much sought after. Multi-targeting ligands that modulate several pathways in the pathogenesis of Alzheimer's present an interesting strategy in this regard. In our search for potential multitargeting molecules, a series of aurones bearing amine and carbamate functionalities were synthesized and evaluated for their anticholinesterase, monoamine oxidase and amyloid-beta (Aβ) inhibitory activities, as well as their drug-like properties including their ability to permeate the blood-brain barrier (BBB) [1]. A trimethoxyaurone bearing piperidine moiety **4-3** was identified as the most promising, drug-like aurone. Bi-directional permeability assay using porcine brain endothelial cells (PBEC) showed **4-3** to be highly BBB permeable and exhibited net influx across the BBB model. To determine if the multipotency of **4-3** can be translated to neuroprotection in a whole organism, **4-3** was evaluated in two *Caenorhabditis elegans* models of neurodegeneration: Aβ-induced paralysis and oxydopamine-induced neurodenegeneration. **4-3** at 25 to 100 μM demonstrated significant protection against both Aβ- and oxydopamine-induced toxicity in the nematode concentration-dependently indicating neuroprotective effect that may be due to its multitargeting feature. Thus, **4-3** represents a current lead for further modification of the scaffold for developing a centrally active neuroprotective agent.

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Concurrent session 4: Natual Medicine

Dietary flavonoids for mood and cognition: evidence from human trials

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Cognitive processes involve multiple mechanisms which interact in complex, ways. Monopharmacological treatments for brain disorders (including cognitive decline and dementia) have had little impact. Over the past two decades there has been a rapid growth in research into the human behavioural effects of nutritional interventions, functional foods and well-characterised dietary supplements. By affecting multiple systems, bioactive nutrients, including widely consumed plant flavonoids may offer a more promising approach. This talk will draw on specific examples fromsystematic assessment of the behavioural effects of functional food interventions including cocoa flavanolsand curcumin from turmeric. A series of double-blind, placebo-controlled crossover studies have been conducted to evaluate the potential for encapsulated flavonoids and flavonoid-enriched fractions of diet to improve cognitive functioning. These include both acute and chronic studies including neurochemical characterisation, as well as evaluation of cognitive outcomes, brain activation (via neuroimaging) and biomarkers of underlying mechanisms. Cocoa flavanols acutely improved effortful mental functioning in a dose-dependent manner whereas chronically they differentially improved mood. Where conducted, brain imaging studies suggest that these effects are linked to improved neural efficiency underpinned by increased blood flow to task-relevant neural structures. A lipidated curcumin preparation improved working memory and reduced fatigue in an older healthy cohort. These studies have demonstrated that dietary flavonoids from a number of sources are capable of improving neurocognitive functioning both acutely and chronically. The mechanisms underlying these effects are not fully elucidated but likely include neurochemical influences on systemic and cerebral blood flow. Preliminary studies in clinical populations suggest that these interventions may be effective in the treatment of age-related cognitive decline.

Explore the potential of cane toad extracts as novel therapy for cancer and/or anxiety

Ming Wei

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Australian cane toad was an imported, eco-disaster that is widely spread and difficult to control. However, a similar toad in China and Asian countries has served as a source of natural medicine for centuries. This presentation looks into the histories of research into toad-related medicines, and explores the potentials of Australian cane toad skin extracts, their primary make-up of compounds and mechanisms of actions in terms of anti-cancer, anti-anxiety and anti-inflammation. Our research indicates that such skin extracts could be used as valuable medicine source, providing a new thinking in the management of the feral pest through toad farming.

Your biologically active natural product may not be a good starting point for drug discovery, even it is an FDA approved drug! Why is this so?

Jonathan Baell

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With increasing access to high throughput screening, academic drug discovery is being accompanied by a plethora of publications that report screening hits as good starting points for drug discovery or as useful tool compounds, whereas in many cases this is not so. These compounds may be protein-reactive but can also interfere in bioassays via a number of other means, many of which may remain unknown, and it can be very hard to prove early on that they represent false starts. We have termed such compounds Pan-Assay Interference Compounds, or PAINS. Examples of such compound cores are shown below. Some of these cores are prevalent in natural products. PAINS were defined from HTS libraries devoid of natural products. So how should one view PAINS-containing natural products in terms of useful biochemical probes or potential therapeutics? This presentation will delve into such issues.[1]

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Repurposing drugs developed from natural products for diabetes and fatty liver disease

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Diabetes is one of the fastest growing diseases worldwide afflicting approximately 380 million people primarily due to a dramatic increase in type 2 diabetes. Despite major investment by pharmaceutical companies in conventional drug discovery pipelines, development of new drugs has failed to keep up with the increasing incidence of type 2 diabetes. Drug repurposing, where existing drugs are applied to a new indication, is gaining momentum as a successful approach to overcome the bottlenecks commonly encountered with conventional approaches. Repurposing takes advantage of available information on the molecular pharmacology of clinical agents, to drastically shorten drug development timelines. This talk will share our recent experience in repurposing existing drugs by targeting fatty acid oxidation (energy expenditure), *de novo* lipogenesis, mitochondrial metabolism, AMPK, CaMKKβ, Foxo1, ER stress, HSP72 and autophagy. The repurposed drugs in our laboratory include beberines (*Diabetes* 57:1414, 2008), triterpenpoids (*Chem Biol* 15:263, 2008; *PlosOne* 9:e10723,2014), rutaecarpines (*ACS Chem Biol* 8:2301,2013; *J Med Chem* 58:9395,2015) matrine (*Bri J Pharmacol* 172:4303,2015; BBA 1852:156, 2015). As well as revealing the new cellular targets for re-evaluation of the molecular mode of action for the treatment of diabetes, this presentation will also discuss relevant cell (*Biochem Pharmacol* 84: 830, 2012) and animal (*PlosOne* 7: e42115,2012) models used in our studies in the screening process for this strategy. Using this approach, we have identified several potential new drugs which could be considered for clinical trials for type 2 diabetes and related metabolic disease.

Concurrent session 5: Translational research and enabling technologies

Molecules to Medicine, developing anti fibrotics for chronic kidney disease

Darren Kelly

OccuRx Pty Ltd, Level 9/278 Collins St, Melbourne VIC 3000

Fibrotech Therapeutics was formed in May 2006 to develop novel drug candidates for the treatment of fibrosis that is prevalent in chronic conditions, such as chronic kidney, heart, and lung disease. The company has since developed a library of novel compounds with enhanced anti-fibrotic actions than that of tranilast, a known anti-fibrotic agent that is approved for the treatment of asthma, allergic rhinitis and atopic dermatitis in humans. The company's lead compound, FT011, has been shown to significantly inhibit the progression of pathological fibrosis in the absence (chronic kidney disease, myocardial infarction) and presence of diabetes (diabetic nephropathy, diabetic cardiomyopathy).
Diabetic nephropathy (DN) is the leading cause of end-stage renal disease (ESRD), where patients would eventually need renal replacement therapy, such as dialysis and kidney transplantation. The use of drug interventions for intensive control of blood glucose and blood pressure, in particular, blockade of the renin-angiotensin system merely delays the progression of DN. Fibrotech envisaged that FT011, currently in clinical development, is a first-in-class drug therapy to treat the underlying pathological fibrosis associated with chronic kidney disease. This program has far reaching social and clinical implications for the community and provides significant economic viability with annual cost per patient per year better than dialysis.

Translation at the Academic-Industry Interface: Case Study Cameos

Jonathan Baell

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Translation at the academic industry interface of drug discovery is challenging, fascination, rewarding. In this presentation and in a series of cameos, I will touch on several successes with which I have had personal experience.

Point of care diagnostics for global health - pushing the boundaries

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Patients in resource-constrained settings continue to have limited access to quality diagnostics due to the need for laboratory equipment and highly trained personnel for most tests. Meanwhile Flow cytometry and bioinformatics are revealing increasing numbers of cell surface molecules and other biomarkers that are of clinical and diagnostic interest in many diseases. Lateral-flow immunochromatography is a proven technology for point-of-care (POC) testing, but the range of its medical applications and market impact has been largely restricted to simple biomarkers such as hormones, antibodies and simple antigens.

Over the past several years we have developed novel approaches in lateral flow that have allowed the development of POC tests that provide viable alternatives to Flow cytometry (CD4 T-cells in HIV/AIDS), enzymatic clinical chemistry (the liver enzyme alanine amino transferase (ALT) in plasma), and other tests in development. These innovations in test development have been coupled with a range of approaches to research translation and commercialisation, greatly expanding the potential medical impact of this robust and proven technology in global health by building on more recent Australian innovation.

Virus-like particle production in the Collaborative Protein Production Facility at CSIRO

George Lovrecz, Carina Joe, Tram Phan, Mylinh La, Louis Lu, Tam Pham and Tim Adams

CSIRO, Manufacturing, 343 Royal Parade, Parkville, 3052, AUSTRALIA

CSIRO's state-of-the-art protein facility has become Australia's only non-commercial ISO9001 and cGMP (APVMA) accredited facility that produces proteins for animal studies and human Phase-I clinical trials. The facility is open for both academic and industrial collaboration to provide access to process development and protein production at best-practice standards.
The Facility employs a range of in-house optimized platform technologies to identify optimum vector-host cell combinations for both transient and stable protein production in traditional or single-use bioreactors. Our short seminar will summarize the recommended steps to use these technologies and provide a quick guide to produce proteins in a cost-effective way.
Also, a case study focussing on the characterization and optimization of the production of a virus-like particle (VLP) will be presented in details. This VLP consists of the Hepatitis B virus surface antigen and with increased immunogenicity it could be the core of a new type of vaccine.

Concurrent session 6: Vaccine ad gene therapeutics

Precise solutions for complex diseases: engaging nanotechnology and immunotherapy

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Advances in technological innovation are enabling the creation of synthetic nanoparticles, with highly defined size, shape and charge properties. Our studies show vaccines using 40-50nm nanoparticles from non-inflammatory materials as antigen carriers induce long lasting and powerful immunity, without inducting the immunosuppressive pathways otherwise elicited to control vaccine induced inflammation, such as TNFR2+ regulatory T cells (Tregs). Moreover, we show such nanoparticles by themselves harbor novel beneficial immune-regulatory non-specific effects (NSEs): rendering lungs resistant to subsequent elicitation of allergic airways inflammation, or promoting clearance of infections such as influenza and malaria.

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Vaccines for prevention and treatment: from influenza to Alzheimer's

Nikolai Petrovsky

Director of Endocrinology at Flinders Medical Centre, Professor at Flinders University and Research Director of Vaxine Pty Ltd

Highly adaptable pathogens including influenza, tuberculosis and HIV continue to circumvent attempts to control them using traditional vaccine approaches. In addition, there is also potential to use vaccines to treat diseases such as cancer and Alzheimer's disease. Hence new technologies are needed to succeed in such challenges. This talk will discuss recent advances including use of alternative antigens, delivery approaches and adjuvants, the latter being an indispensible but poorly understood tool for maximising vaccine efficacy.

Development of Novel Genetic Therapies for Haemoglobin Disorders

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The β-thalassaemias, sickle cell disease (SCD) and haemoglobin E (HbE) disease represent the most important haemoglobinopathies from a clinical point of view, causing severe morbidity and mortality worldwide. Current standard of care involves life-long regular blood transfusions and iron chelation therapy to reduce iron toxicity.

The transfer of gene-corrected autologous haematopoietic stem cells (HSCs) could provide a therapeutic alternative, as recent lentiviral gene therapy trials have demonstrated. The greatest caveat in the use of integrating lentiviral vectors is in the inability to control the site of integration, which can potentially cause genotoxicity. This issue has driven the search for safer gene therapy approaches.

One possible solution is to target the integration of a therapeutic gene into a genomic "safe harbour" site that supports long-term transgene expression. Alternatively, genome editing could be used to correct patient HSCs *ex vivo*. Ideally, correction of the β-globin gene in HSCs could be achieved through homology-directed repair (HDR), resulting in the production of healthy erythrocytes. Several studies including work from our group have shown that the human β-globin locus is amenable to genome editing. However, technical limitations and safety concerns need to be overcome for this novel approach to become clinically feasible. Here, we highlight recent developments and important new directions in β-thalassaemia and SCD gene therapy.

Virus-like Particle Engineering for Therapeutic Delivery

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Biomimetic and bio-derived nanoparticle delivery vehicles are proving to be exciting technologies for the future of therapeutic delivery. Evolved to contain and protect nucleic acids and proteins viruses exhibit a wide variety of cell uptake mechanisms designed to delivery sensitive cargo to the cytosol or nucleus of cells. The self-assembled protein shells of viruses can be repurposed into engineering virus-like nanoparticle (VNPs) for use in various bionanotechnology applications such as drug delivery, molecular imaging and designer vaccines. VNPs can be employed to address the main challenges in nanoparticle therapeutics: cargo-loading, cell delivery and efficient uptake. We are developing two VNPs with very different structures and assembly mechanisms. I will present recent progress in my group to elucidate the subnanometre resolution of recombinant Bluetongue virus core-like particles and their subsequent engineering for potential therapeutic delivery. In addition I will highlight the utility of native capsid protein features of Murine polyomavirus-like particles in directing cell-receptor targeting and enabling endosomal escape. Our results point to the great potential of VNPs in bionanotechnology and the advantages in exploiting their inherent properties to meet some of the challenges facing nanomedicine. Our work also highlights the importance of detailed structural analysis of VNPs in validating their molecular organisation and the value of such analyses in aiding their design and further modification.

Concurrent session 7: Stem cell therapeutics

Harnessing pluripotency: novel antibodies for human stem cell biology.

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Human embryonic stem (hES) cells and human induced pluripotent stem (hiPS) cells, collectively termed human pluripotent stem cells (hPSCs), can indefinitely self-renew and differentiate into essentially all adult cell types. This renders them potential sources of material for a wide range of clinical applications. However, there remains a real need to develop new tools that will safely enable both large scale purification of live hPSC cultures, as inputs to clinically relevant differentiation assays, and the stringent removal of residual tumorigenic pluripotent cells from end-point cell populations following differentiation1. We have previously reported the use of a FACSbased immunotranscriptional profiling system to characterise multiple hPSC lines and using this have identified all proteins likely to be expressed on the surface of pluripotent cells that switch off upon differentiation2-4. To generate tools for detecting cell-surface proteins on viable hPSCs we selected candidate genes identified from our published immunotranscriptional profiling of hPSCs. Following immunisation with target antigens, hybridoma cultures were screened by robotic solid-state antigen array analyses for detection of the corresponding immunogen and then via high-throughput flow cytometry to confirm capability for detecting live hPSCs. Here we report the generation of a new panel of 7 monoclonal antibodies (mAbs) that efficiently detect these known proteins on the cell surface of live and fixed hPSCs derived from both the embryonic inner cell mass (hESCs) and somatic cell reprogramming (hiPSCs). We anticipate that these novel antibodies generated to known antigenic targets will be valuable tools for enabling further investigation of human pluripotency and should also facilitate the development of strategies for quality control of hPSC-derived cell populations destined for clinical use.

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Rapid optimization of lineage commitment and media formulation using microfluidic bioreactors

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Dietary Polyunsaturated Fatty Acids Modulate the Adipose Secretome to Modify Mammary Stem Cell Self-Renewal

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Rationale: Chronic inflammation associated with dysfunctional adipose partly triggers the obesity-associated metabolic syndrome, a modifiable risk factor for cancer. We tested the hypothesis that dietary ω3 fatty acid supplementation in obesity favorably rebalances the adipokine milieu to reduce mammary stem cell self-renewal.

Methods: Male F344 rats were fed a high fat Western blend control diet (ω6:ω3 fatty acid ratio = 20:1) or high fat menhaden fish oil diet (ω6:ω3 fatty acid ratio = 2.5:1) x 24 wks. Conditioned media from omental explants in long term 3-D culture was assayed with the Proteome Profiler Cytokine XL Array. The rate of primary normal human breast stem cell self-renewal was quantified after 10 days of incubation in adipose conditioned media from rats fed high rat Western blend or menhaden fish oil diets.

Results: After 24 week Western blend rats weighed 444.8 ± 14.2 gm (mean±SD), fish oil diet rats weighed 436.8 ± 10.9 gm (p=0.66). Volume distribution of individual adipocytes was reduced by 26% (p<0.001) in the ω3 diet group (mean= 0.94 x106 μm3, n=500 cells) compared to the Western Blend diet group (mean= 1.27 x106 μm3, n=500 cells). The adipose ω6:ω3 PUFA ratio was reduced to 3.2:1 in the fish oil compared to 26.4:1 in the control Western blend diet rats (p<0.001). The fish oil diet shifted the cytokine proteome in adipose conditioned media from the Western blend diet causing a 4-fold increase in secreted adiponectin and significant decreases in pro-inflammatory factors such as leptin, resistin, and lipocalin. Media conditioned with rat adipose tissue increased normal human breast stem cell self renewal in Western blend 61.6 ± 3.7% compared to fish oil adipose increase of 11.4 ± 4.3% (p<0.001) compared to no adipose conditioned media.

Conclusions: A dietary or supplemental intervention that modifies the ω6:ω3 PUFA ratio quenches adipose inflammation and rebalances the adipokine milieu. Adipose derived paracrine and endocrine signals play a role in control of human mammary stem cell self renewal and are a component driving obesity associated carcinogenesis.

The role of B-catenin in muscle stem cell function and dysfunction

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Muscle repair is mediated by a small population of adult muscle stem cells called satellite cells. Muscular dystrophies (MD) are devastating diseases with few therapeutic options. MD involves an imbalance between degeneration and regeneration; manipulating endogenous satellite cell activity may thus provide new therapeutic avenues. We are investigating the role of canonical Wnt signalling in muscle repair. Wnt signalling induces satellite cell/myoblast differentiation *in vitro*. In contrast, excessive upregulation of Wnt signaling with aging has been reported to promote fibrosis via both activation of muscle-associated fibroblasts and myogenic-fibrogenic conversion of satellite cells [1]. We developed an *ex vivo* CRISPR model to investigate the role of beta-catenin in adult mouse primary myoblasts. Deletion of beta-catenin dramatically inhibited differentiation and delayed induction of the myogenic transcriptional program. This result is in agreement with a recent beta-catenin null mouse model [2], and contradicts a previous *in vivo* study showing no function for beta-catenin in adult myoblasts [3]. Using RNA-seq and ChIP-seq analyses we have defined the transcriptional program induced by Wnt-beta-catenin signalling in myoblasts and developed a model in which beta-catenin cooperates with myogenic regulatory factors at non-TCF/LEF sites to promote myogenic differentiation. In contrast beta-catenin may act via TCF/LEF sites to induce fibrogenic genes. This work paves the way for differential inhibition of beta-catenin-cofactor complexes to curtail pro-fibrotic signalling, whilst maintaining essential pro-differentiation functions.

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Concurrent session 8: Computational approaches

Capturing flexibility in structure-based design: from drugs to sugars and back again

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Computational approaches to drug formulation

David Chalmers

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Drug formulations are complex mixtures of chemical components. Once a formulation reaches the gastrointestinal (GI) tract, it is diluted and comes into contact with bile and digestive enzymes which can modify the formulation producing a mixture that is difficult to study experimentally. This presentation will discuss our use molecular dynamics to develop coupled molecular dynamics and experimental models to investigate the colloidal structure of drug formulations and how these formulations behave within the gastro-intestinal tract.

Circumventing the stability-function trade-off in protein engineering

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The favorable biophysical attributes of non-antibody scaffolds make them attractive alternatives to monoclonal antibodies. However, due to the wellknown stability-function trade-off, these gains tend to be marginal after functional selection. A notable example is the fibronectin type III (FN3) domain, FNfn10, which has been previously evolved to bind lysozyme with picomolar affinity (FNfn10-α-lys), but suffers from poor thermodynamic and kinetic stability. To explore this stability-function compromise further, we grafted the lysozyme-binding loops from FNfn10-α-lys onto our previously engineered, ultra-stable FN3 scaffold, *FN3con*. The resulting variant (FN3con-α-lys) bound lysozyme with a markedly reduced affinity, but retained high levels of thermal stability. The crystal structure of FNfn10-α-lys in complex with lysozyme revealed unanticipated interactions at the protein-protein interface involving framework residues of FNfn10-α-lys, thus explaining the failure to transfer binding via loop grafting. Utilizing this structural information, we redesigned FN3con-α-lys and restored picomolar binding affinity to lysozyme, whilst maintaining thermodynamic stability (with a thermal melting temperature two-fold higher than that of FNfn10-α-lys). FN3con therefore provides an exceptional window of stability to tolerate deleterious mutations, resulting in a substantial advantage for functional design.

Design of Therapeutic Peptides Using the Resonant Recognition Model

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Peptides have been used in wide range of applications in medicine and biotechnology, supporting increase in therapeutic peptide research. The main disease areas for therapeutic use of peptides are metabolic diseases and oncology, with also usage for infectious diseases and inflammation. Currently, rational design of therapeutic peptides is based on known protein tertiary and secondary structures, as well as physicochemical properties of natural peptides [1]. However, designing bioactive peptides from natural proteins and their structures is challenging, as 3D structure is not the only parameter describing specificity of protein functions/interactions. The other challenge is to mimic natural protein 3D structure with shorter peptides. Thus, there is a need for completely different approach to understand selectivity of protein functions/interactions and consequently design more potent therapeutic peptides.

Here we present the Resonant Recognition Model (RRM) [2-4], which is new approach to analyses of protein structure/function and is based on the finding that certain periodicities in distribution of free electron energies along the protein are crucial for the biological function/interaction of proteins. Once this characteristic periodicity (frequency) of biological function of the protein has been identified, it is possible to design *de novo* proteins and/or shorter peptides having only desired characteristic frequency(ies). Consequently, such designed proteins/peptides are proposed to have desired biological function/interaction. The RRM approach has been already successfully applied and experimentally tested in design of: fibroblast growth factor analogue [5], HIV envelope protein analogue [6] and peptides related to oncolytic function [7-9]. Future applications of RRM approach [10], particularly in oncology, will be discussed here.

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Concurrent session 9: Drug delivery

Lipid based delivery systems for unmet medical needs

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Lipids can rival polymers for diversity of form and function, and have an inherent toxicity advantage evidenced by the success of translation of liposomes into the clinic. The full utility of liposomes has been far from realised and the potential use of liposomes in addressing traumatic brain injury will be presented. More complex lipid nanoassemblies also offer new functional advantages over the humble liposome for treatment of diseases where on-demand pulsatile release behaviour may be beneficial, through reversible lock and key behaviour enabling multidosing after a single administration in diseases such as diabetes and macular degeneration.

mRNA delivery using nano drug delivery systems for intractable diseases and regenerative medicine

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Gene therapy is defined as introducing genetic information for therapeutic purposes. Besides conventional strategies of protein replacement therapy, gene therapy may have wide application including regenerative medicine by introducing “therapeutic” signaling molecule(s). In addition, cell therapy combined with ex vivo gene introduction is also a promising field. Viral and non-viral vectors have been widely used to express transcription factors. However, the former still have safety concerns, including issues related to insertion into the host genome, while the transfection efficiency of the latter remains quite low, especially in post-mitotic cells. Direct delivery of messenger RNA (mRNA) into cells should be highlighted as an emerging technology in this context, since it would directly achieve the expression of proteins of interest without the above concerns associated with viral and non-viral vectors.

Despite the fact that mRNA delivered in vivo are susceptible to highly active RNases that are ubiquitous in the extracellular space, we established a drug delivery system using polyplex nanomicelles to transport mRNA into target cells by preventing its degradation. Polyplex nanomicelles are based on the self-assembly of polyethylene glycol (PEG)-polyamino acid block copolymer, possessing a PEG outer layer and mRNA-containing core. This system provides excellent in vivo stability of mRNA under physiological conditions. Furthermore, the stealth property provided by the polyplex nanomicelle surface, which is composed of dense PEG palisades, effectively prevents the inflammatory responses that are often caused by unfavorable immunogenicity of mRNA.

In this presentation, I present the chemical basis of the polyplex nanomicelle system for in vivo mRNA delivery. Then, our trials for treating various diseases will be presented for discussing the strategy of mRNA-based therapeutics.

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Engineering endosomal escape using pHlexiparticles

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Therapeutic interventions for the treatment of burn injuries and fibrotic skin disorders

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Scars are both aesthetically but more importantly functionally inferior to normal skin, resulting in lasting psychological and physical side effects. Scar formation following injury is a type of fibrosis, where the dermal layer of the skin exists of a highly dense and crosslinked region of collagen fibers. Skin fibrosis and scarring is a significant clinical problem. Every year, there are over 48 million operations in the USA alone that result in scars. In addition to these surgical scars, there are approximately 11 million burn injuries globally, resulting in 300,000 deaths per year. Currently there are no approved drugs which can reduce scarring or fibrotic tissue formation. My talk today will focus on a number of therapeutic interventions for addressing this issue. The first using magnetic nanoparticles for 'magnetofection' of skin cells to move cell populations with a magnetic field, the second involving the delivery of antifibrotic drugs from within a polymeric nanoparticle system and thirdly our work involving a lysyl oxidase (LOX) inhibitor to modify collagen crosslinking in a scar like environment (Figure 1).

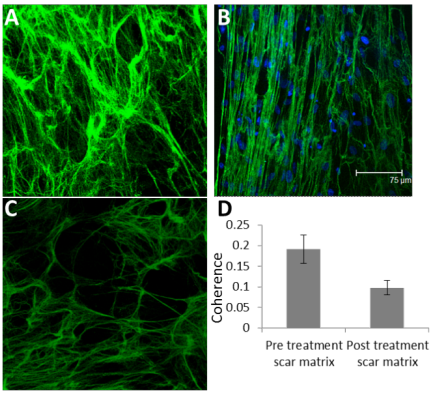

Figure 1. LOX inhibition alters matrix deposition in scar fibroblasts. Scar fibroblasts in culture deposit a dense striated collagen I matrix (B) compared to more normal basket weave structure from skin cells (A).Lox

inhibition promotes a more normal structure (C)

Concurrent session 10: Mechanisms of diseases and drug development

Novel pharmacological strategies for the treatment of chronic obstructive pulmonary disease and its comorbidities

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Introduction: Reactive oxygen species (ROS) are a family of highly reactive molecules that are produced by a variety of cell types in the lung in response to chemical and physical agents in the environment. It is well known that ROS are critical in host defence as they kill invading pathogens, but that their excessive accumulation in the lung results in oxidative damage. Oxidative stress, which is defined as the persistent overproduction of ROS that overwhelms endogenous antioxidant defence systems, has been implicated in both acute (e.g respiratory virus infections, exacerbations of asthma and COPD) and chronic (e.g. COPD) lung diseases.

Aims & Methods: To determine whether inhibiting oxidative stress and ROS production may be a novel way to treat acute and chronic lung diseases using clinically relevant models of lung disease.

Results: We have shown that targeting oxidative stress with the Nox2 oxidase inhibitors and ROS scavengers, apocynin and ebselen can ameliorate influenza A virus (IAV)-induced lung inflammation and pathology, cigarette smoke-induced lung inflammation and acute exacerbations of COPD (AECOPD). In addition, we have found that treating mice with apocynin reduced cigarette smoke-induced skeletal muscle wasting in mice suggesting that this strategy can be useful in treating comorbidities associated with COPD.

Conclusion: Targeting oxidative stress may be a novel strategy to treat both acute and chronic lung diseases.

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Non-kinase targets of protein kinase inhibitors

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Mitogen-activated protein kinase (MAPK)-activated protein kinase 2 (MK2) is a checkpoint kinase regulating DNA damage response, a mechanism that is crucial for survival of cancer cells. Thus, MK2 has been identified as a promising avenue for targeted cancer therapeutics. Majority of reported MK2 inhibitors bind into the ATP pocket of the targeted kinase. Because MK2 has high affinity for ATP, the efficacy of ATP-competitive MK2 inhibitors is compromised by intracellular ATP concentration. This results in low biochemical efficiency index and compounds that do not possess drug-like properties.

We have addressed this issue by developing a library of non-ATP competitive MK2 inhibitors. The novel MK2 inhibitors exhibited potent antiproliferative efficacy in glioblastoma cells and primary gliomaspheres, without affecting the viability of non-malignant cells.[1,2] Intriguingly, we also discovered an unexpected non-kinase target for this class of compounds. Chemistry development and biological mechanism of action of these new compounds will be presented. I will also discuss how in-depth biological understanding of a molecular target is necessary in the early stages of the drug discovery and how mechanism of action can determine later success or failure of the emerging drug candidates.[3]

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Synthesis of Novel Anti-Metastatic Agents via Inhibition of of Cell-Surface Sialylation

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Sialic acid occupies the terminus of the oligosaccharide chain of several cell surface glycoconjugates and thus plays a critical role in cell-cell recognition, adhesion and inflammation. Correlations have been discovered between sialylation and tumour malignancy, B lymphocyte activation and immune regulation, with aberrant sialylation now a marker for several diseases [1]. Tumours have hijacked these sialylation processes to facilitate tumour metastasis and evade immune detection, exhibiting up to 30% higher degree of sialylation than normal cells.

Accordingly, a number of potent ST inhibitors have been developed to explore STs as a new cancer target [1]. However many of these inhibitors are considered too hydrophilic to diffuse across the cell membrane or are broad-spectrum inhibitors that may result in off-target effects. There are 20 different types of human STs each with a defined biological function. In order to proceed to the clinic it is essential to develop highly potent, selective, cell permeable ST inhibitors. Herein we present our work towards the structure-based design and synthesis of selective, cell permeable inhibitors of ST3, ST6 and ST8 inhibitors with potential applications as antimetastatic agents in pancreatic, ovarian and breast cancer [2].

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Construction of Bioactive Compounds on Purine Isosteric Scaffolds

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Purines represent a class of the most ubiquitous and functionally diverse nitrogen-containing heterocyclic compounds in nature. Being in the focus of medicinal chemist interests for many years, the purine scaffold has become a privileged structure in the drug discovery and development. Isosteric modifications of this heterocyclic skeleton further increase diversity and chemical space of compounds targeting druggable purinome.

We have been developing new synthetic methods for the preparation of bioactive compounds using scaffolds isosteric to purine. The main area of our research program are purine isosteres with a nitrogen atom in the position 5 of the purine heterocyclic system: pyrazolo[1,5-*a*][1,3,5]triazines, 1,2,4-triazolo[1,5-*a*][1,3,5]triazines, and imidazo[1,2-*a*][1,3,5]triazines.1 A number of efficient and complementary to each other approaches were developed for the synthesis of these bioactive molecules by our group. Among them are highly potent in nanomolar range anti-leukemic compounds, which selectively cause apoptosis in leukemic cells and do not affect normal cells even at concentrations hundreds time higher. We designed and prepared highly potent xanthine oxidase inhibitors many folds exciding activity of bioisosteric to hypoxanthine drug, allopurinol, which is a gold standard of gout therapy. The synthesis and biological activity of these and other examples are discussed in this presentation.

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Aptamers as therapeutic modalities for the treatment of breast cancer brain metastases

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Metastatic brain tumours occur in approximately 25% of all cancer patients following primary malignancy treatment. Survival rates for patients with brain metastases have increased only slightly in the last twenty years. Protected by the body's most formidable barrier, the blood brain barrier (BBB), treatment options for patients is limited and thus prognosis is poor, with survival time measured in months. A promising new approach toward by-passing this barrier, is to hijack active transport mechanisms present on the endothelial cell membranes, in particular the transferrin receptor (TfR). Nucleic acid based aptamers, also known as chemical antibodies, are ideal for this purpose given the ability to generate them against a vast range of targets, their stability, and safety profile. This study has generated a bi-functional aptamer that binds to two different targets, the transferrin receptor on the blood brain barrier and EpCAM, a marker of metastatic cancer cells, which can transcytose the BBB by targeting the TfR and specifically deliver a cytotoxic payload to the EpCAM positive tumours, thus minimising cytotoxicity to healthy brain cells. To achieve this, a 14-mer DNA aptamer against the TfR was joined to a 19-mer DNA aptamer targeting EpCAM, a glycoprotein overexpressed in a number of solid tumours, including breast cancer, with a high incidence of metastasing to the brain. The specificity and selectivity of these aptamers was confirmed against a number of cells lines expressing the TfR, EpCAM or neither protein, using flow cytometry and confocal microscopy. The ability of this aptamer to enter the brain in a living system was also demonstrated using an in vivo mouse model, with the results showing specific transcytosis into the brain within 10 minutes following tail vein injection. Intercalation of the common chemotherapeutic doxorubicin showed no influence on aptamer specificity, with the conjugate specifically internalised within the targeted cells following one hour incubation. The results of this study confirm initial proof of concept that aptamers can be effective as therapeutic modalities for the treatment of neurological disorders and demonstrate the great potential this bi-functional aptamer doxorubicin conjugate has for the specific treatment of brain metastases, which will improve patient survival and quality of life, in addition to the possibility of mitigating the neurotoxic effects on healthy brain tissue.

Concurrent session 11: Diagnostics, sensors and imaging

Nanozyme biosensors: from molecular diagnostics to bacteria, virus and cancer detection

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Non-toxic quantum dots and Magnetic nanoparticles as bio-imaging agents

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Solution synthesis is a powerful method for the formation of uniform sized and properties.1-2Strategies for the formation of magnetic ironnanoparticles and through chemical synthesis will be outlined. The mechanisms have been elucidated from in-situ XRD and HRTEM. The superparamagnetic properties of these materials willand biomedical applications magnetic nanoparticles for MRI contrast will bediscussed.[1,2] The synthesis optical properties and applications of low toxicity silicon and germanium quantum dots for bio-medical imaging will also be discussed.

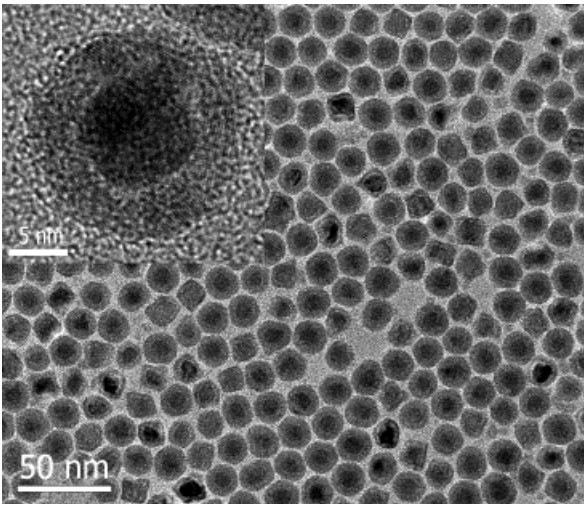

Fig. 1. TEM image of iron core/iron oxide shell nanoparticles.

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Salivary diagnostics for the detection of cardiovascular diseases and cancers

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It is now becoming evident that there is a strong association between oral and systemic diseases. Human saliva has gained attention as the diagnostic fluid of the future. Human saliva functions as a plasma ultra-filtrate and contains 2,340 proteins that are either transported across blood into salivary glands or produced by the salivary glands. The collection of saliva is less invasive compared with taking a blood sample and as a result saliva is very accessible to both patients and clinicians. Saliva is also a medium that is ideal for large population-based screening and potentially provides healthcare systems a more economical approach to detecting diseases within the community.

Within our team, we are using saliva as a biological matrix to detect ischemic heart disease and heart failure. We used AlphaLISA® technology to quantify C-Reactive Protein levels in saliva samples collected from controls and cardiac patients. The mean CRP levels in the saliva of controls was 285 pg/mL and in cardiac patients was 1680 pg/mL ($p<0.01$). Analysis of CRP concentrations in paired serum and saliva samples from cardiac patients gave a positive correlation ($r2 = 0.84$, $p < 0.001$) and the salivary CRP concentration capable of distinguishing healthy from diseased patients. Similarly, salivary NT-proBNP levels in the healthy controls and HF participants were <16 pg/mL and 76.8 pg/mL, respectively. The salivary NT-proBNP immunoassay showed a clinical sensitivity of 82.2% and specificity of 100%, positive predictive value of 100% and negative predictive value of 83.3%, with an overall diagnostic accuracy of 90.6%. Galectin-3 levels were also elevated in saliva from HF pateints (n=51) compared with controls (n=63) with an area under the curve (AUC=0.73).

Saliva is in close proximity to head and neck cancers (HNC), especially oral cavity cancers and by analysing salivary DNA methylation and miRNA signatures, we have been able to detect these cancers. DNA methylation, addition of methyl groups to CpG promoter sites and the changes of

methylation is a hall mark of cancer. We have identified common tumour suppressor genes to be methylated in saliva collected from HNC patients compared with controls. Using a sensitive methylation-specific polymerase chain reaction (MSPCR) assay to determine specific methylation events in the promoters of RASSF1α, $p16^{INK4a}$, TIMP3 and PCQAP/MED15 were higher in HPV-negative HNSCC patients (n = 88) compared with a normal healthy control group (n = 122) (sensitivity of 71 % and specificity of 80 %). Conversely, DNA methylation levels for these genes were lower in HPV-positive HNSCC patients (n = 45) compared with a normal healthy control group (sensitivity of 80 % and specificity of 74 %), consistent with the proposed aetiology of HPV-positive HNSCC. A panel of nine salivary micro RNA (miRNA) markers has demonstrated a sensitivity of 87% and a specificity of 75% (AUC = 0.90) when discriminating saliva collected from patients (n=100) vs saliva collected from controls (n=60).

These data support the idea that saliva is an ideal biological medium to detect both oral and systemic events. Further research is warranted in translating these findings into a clinical setting.

From cytokine sensing to cell based therapy

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Cytokines secreted from cells play critical roles in tissue repair, cancer development and progression. Unfortunately, probing what cells "see", and what they secrete as they respond in real time to the surrounding signals is still a major challenge. The ultra-low concentration of cytokine (in the pM range), and extremely dynamic, transient cytokine secretion process make cytokine quantification even difficult. Herein we developed a simple and sensitive on cell surface ELISA assay (OnCELISA) to identify and select high cytokine-secreting cells. This cytokine analysis platforms that (1) enables a nuanced characterization of individual immune cells; (2) is capable of quantitative analysis of cytokines secreted from each cell based on fluorescence, (3) is sensitive enough to probe physiologically significant cytokines such as IL- 6, IL-1β, (4) does not significantly affect the functioning of assayed cells which can be cultured after selection, and (5) is able to sort the high secreting cells by magnetic field. The sensitivity of OnCELISA is 0.1 pg/mL with the linear range of 0.1-1000 pg/mL. This technology is applicable to develop the cell based therapy for a range of physiological and pathological conditions such as tumours.

Concurrent session 12: Mechanisms of diseases and drug development

A new therapy to promote myelination and improve neurological outcomes following fetal growth restriction.

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Background and Aims: Due to poor cerebral myelination, babies born after intrauterine growth restriction (IUGR) have an increased risk of neurodevelopmental impairments such as cerebral palsy (CP). In pregnant rodents we know that IUGR results in myelination deficits in the offspring due to delayed maturation of oligodendrocytes (Tolcos et al., 2011); however, the mechanisms that underpin this delay are not fully known and there is no corrective treatment. Here, we propose that alterations in thyroid hormone (TH) uptake and signaling is the cause of the delayed oligodendrocyte maturation and reduced myelination, and that DITPA (3,5-diiodothyropropanoic acid), a novel analogue of TH that does not require the transporter monocarboxylate transporter-8 (MCT8) for cellular uptake, can correct the myelination deficit in IUGR.
Methods: At embryonic day 18 (term=22 days), pregnant rats underwent bilateral uterine vessel ligation to generate IUGR pups (n=5); sham surgeries generated controls (n=5).
Brains were collected at postnatal day 2, (P2), P7 and P14 and used to assess: (i) gene expression of *MCT8*, TH receptors alpha and beta [*TRα*, *TRβ*], and TH enzymes (*D2, D3*) in white matter brain regions; (ii) density of myelinated fibres and mature oligodendrocytes. A second cohort of control and IUGR rat pups (n=3-5) were then treated with DITPA (0.5mg/100g body weight; i.p) from P1-6 and the brains were collected at P7 for assessment of myelination.
Results: In IUGR vs control neonates there was a significant decrease in: (i) myelinated fibres in the cerebral white matter at P7 and P14; (ii) the density of mature oligodendrocytes at P14;
(iii) gene expression for *MCT8* and *TRα* at P7, with no effects on *D2, D3* or *TRβ* expression at P7. Treatment of IUGR neonates with DITPA restored myelination to control levels at P7.
Conclusion: These findings confirm our hypothesis that reduced myelination arises because of decreased MCT8 and TRα in the white matter of the IUGR brain, and it can be corrected by postnatal treatment

with DITPA - potentially a new therapy to improve longterm outcomes for human babies born IUGR.

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Occurrence of G-quadruplex DNA in fibrosis and astrogliosis

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Synthesis and Pharmacological Evaluation of Novel Positive Allosteric Modulators of the M1 Muscarinic Acetylcholine Receptor

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Positive allosteric modulators (PAMs) of the M_1 muscarinic acetylcholine receptor (M_1 mAChR) are a promising strategy for the treatment of the cognitive deficits associated with diseases including Alzheimer's and schizophrenia. Benzoquinazolinone **1** (**Fig. 1**) is a positive allosteric modulator (PAM) of the M_1 mAChR, which is significantly more potent than the prototypical PAM, 1-(4-methoxybenzyl)-4-oxo-1,4-dihydroquinoline-3 - carboxylic acid (BQCA). We have designed, synthesized and pharmacological evaluated three novel families of M_1 mAChR PAMs that are based on an arylpyrimidinone,[1] 4-phenylpyridinone[2] and 4-phenylpyrimidinone scaffold (**Fig. 1**), respectively. Particular attention was paid to the importance of the tricyclic scaffold of compound **1** for the activity of the molecule. The most active compounds exhibited comparable binding affinity to the literature compound **1**, but markedly improved positive cooperativity with acetylcholine, and retained exquisite selectivity for the M_1 mAChR. Furthermore, our pharmacological characterization revealed ligands with a diverse range of activities, including modulators that displayed both high intrinsic efficacy and PAM activity, those that showed no detectable agonism but robust PAM activity, and ligands that displayed robust allosteric agonism but little modulatory activity. Thus, these results offer an attractive starting point for further lead optimization as well as preliminary *in vivo* studies, which will allow us to explore the relationship between *in vivo* efficacy and *in vitro* parameters of M_1 mAChR PAMs.

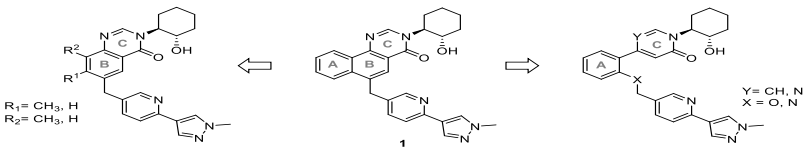

Figure 1. Overview of the chemical structures and design strategy behind the novel M_1 mAChR PAM scaffolds; the Merck compound (middle) provided the starting point for the design of the arylpyrimidinone (left), 4-

phenylpyridinone and 4-phenylpyrimidinone (right) based derivatives.

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Photo-modulated Ocular Drug Delivery of Biomacromolecular Therapeutics

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Age-related macular degeneration (AMD) is the leading cause of blindness in Australia today.[1] The only way to save vision requires ongoing invasive monthly injections of biomacromolecular anti-angiogenics to the eye.[2] A drug delivery reservoir which enables sustained 'on-demand' release of therapeutics would be beneficial, since it will significantly reduce the number of injections into the back of the eye.[3] Here we report the fabrication of a photo-responsive drug delivery system based on a thermo-responsive polymer containing light sensitive nanoparticles and therapeutic payloads(Fig. 1). Released biomacromolecules exhibited above 85% retention of their biological activity. The formulation did not show *in vitro* toxicity to ocular cells, and can be injected subconjunctivally through a 30- gauge needle. Due to its minimum toxicity, tuneable drug release rates and high versatility, this photo-modulated drug delivery system has a potential to improve the treatment of ocular diseases.

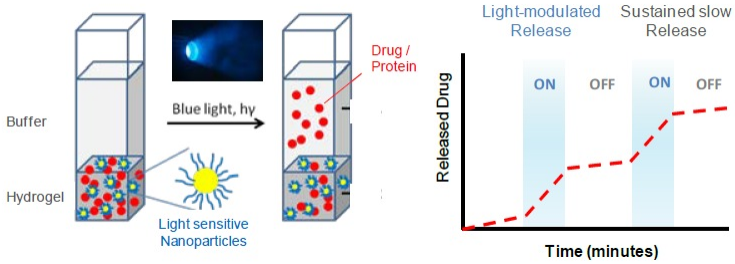

Figure 1. Schematic representation of photo-modulated drug/protein release from the hydrogen, and the release profile of the drug into the buffer in the absence (OFF) and presence of light (ON).

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POSTER SESSION

Asymmetric silica nanoparticles as a vaccine platform: Influence of shape on Immunological responses

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Abstract: Asymmetric nanoparticles with variouscompositions haveattracted much attention in diverse fields. Very recently, asymmetric mesoporous silica nanoparticles (MSN) haveemerged as an interesting family of nanocarriersfor therapeutic delivery.1However, the asymmetric MSN reportedto date have relativelysimple architecture with small pore size. Moreover, there is little report on the interaction of asymmetric MSNs and immune cells. One unique advantage ofMSN for biomedical application istheirlarge pore size for the delivery ofbiomolecules2such as vaccine proteins. It remains a challenge to construct novel asymmetric MSN with large pore sizes andenhanced interaction with immune cells.

Herein, we reported the synthesis of head-tail MSN (HTMSN) which consistsof two parts: asolidsilica or porous silica head and, a dendritic tail with large pores. The HTMSNconsist ofdual pores (large and small pore size) both in head and tail region. The tail length of HTMSN can be successfully adjustedby changing the tetraethyl orthosilicate (TEOS)and head amount. The advantage of HTMSN was shown byinteraction of nanoparticles with antigen presenting cells (APC). The uptakeof HTMSNintoAPC was higher compared with dense solid silica spheres. In addition, HTMSN showed improved CD40 and CD86 expression on dendritic cells and macrophages, indicating that the HTMSN havehighly activating immune response. This work sheds light on thedesign of novelasymmetric MSN with great potential ascarriersand adjuvantsforvaccine delivery.

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Zinc doped ferrite nanoparticles for magnetic resonance imaging

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Ferrite nanoparticles(FNP)more specifically spinel ferrite have been widely utilized in biomedical diagnostics, therapeutics and a broad spectrum of other fields.1-3However, forbiomedical application FNP must be biocompatible, have well controlled physicochemical propertiesandsuitable magnetic features. We herein report the synthesis of monodispersed zinc doped ferrite NPby hydrothermal co-precipitation methodand further optimized theconcentration of zinc dopant for magnetic resonance imaging (MRI). Physical properties of the FNP were evaluated by x-ray diffraction analysis, semiconductor quantum interference device measurement, transmission/scanning electron microscopy, Brauner Emmett teller surface area analysis and dynamic light scattering techniques. MRI investigation of the optimized zinc doped FNP(Zn0.4Fe2.6O4) showed enhanced magnetization (108.4 emu/g) and improved T2 relaxation contrast when compared to undoped NPat similar concentrations. Furthermore, x-ray computed tomography was also assessed for the optimized zinc doped FNPin order to evaluate the multimodal diagnostic potential. In vitro studies with monocytes and lymphocytes cell lines revealed minimal cytotoxic effects up to 100μg/ml zinc ferrite nanoparticle concentration. Overall, the FNPbased contrast agent developed in this study is a promising platform for MRI diagnostic contrast agent development.

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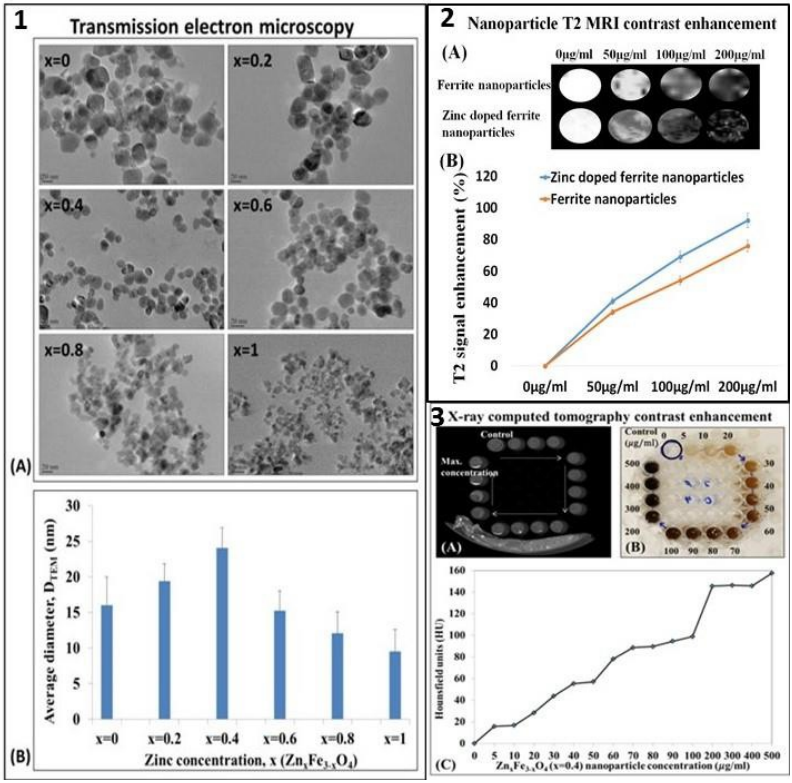

Designing Turn-on Fluorescent Probes for Nitroreductase

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Nitroreductase (NTR) is a bacterial enzyme that catalyzes the chemical reduction of nitroaromatics to aromatic hydroxylamines and anilines. NTR is of particular interest due to its role in drug resistance in bacteria as well as its possible role in enzyme prodrug therapy. Thus, strategies that allow us to image NTR and its activity via fluorescence are highly sought after.

We initially took a previously reported turn-on fluorescent probe1 based off the chromophore resorufin in order to design our own self-immolative fluorescence-quenching handle. When testing these probes with the NTR enzyme we revealed that the resorufin fluorophore itself could be reduced by the enzyme to produce a non-fluorescence entity.

We instead used the fluorophore 4-carboxy Tokyo Green2 as the basis of our fluorescent probe (**Figure 1**.). From this we were able to develop a stable probe that when exposed to NTR, resulted in a 10 fold increase in fluorescence. We are currently working on developing new handles for our probe that can be efficiently reduced by the enzyme.

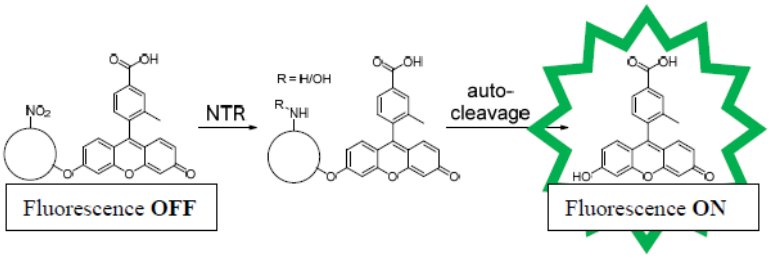

Figure 1: Design of NTR probe

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Hit-to-lead optimization: Single agents for the treatment of Chagas disease

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In 2014, GSK performed a high-throughput-screen of 1.8 million compounds against threekinetoplastid parasites. This data was published as an open source in an effort to encourageresearch and drug development for these neglected diseases (1). A simple arylthioether compound (compound **1**) was found to have desirable activity against Trypanosoma cruzi (theparasite responsible for Chagas disease) and was selected as the hit compound for this project. The initial investigation led to thediscovery of an even more potent compound, with a superior pIC50 of 7.5 (compound **2**).

This class of compoundsshowed promising results in acute in vivo efficacy studies and was observed to clear parasitaemiaatgreater than 70% in infected mice at only 30 mg/kg per day. However, several issues have beenidentified for this chemical series, such as toxicity and low exposure. A full toxicity study wasundertaken and several alerts were identified that relate to CNS and cardiovascular toxicity. Theseinclude CB1/2, GABA-A, PDE3A, nicotinic and hERG. In order to address these concerns, futureanalogues have been focused to decrease toxicity and increase exposure. This will be achieved byexploring lipophilicity, solubility and increasing microsomal stability.

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Neem plant protein based nano-biopesticides and targeted cancer nanotherapy

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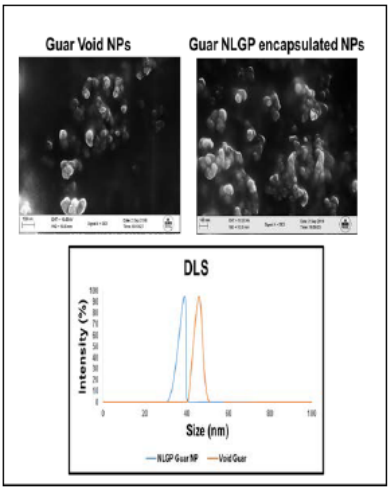
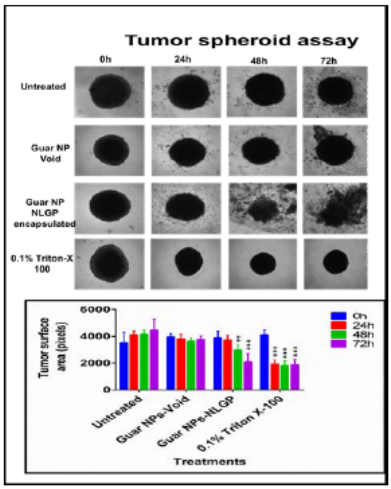
Innumerous use of synthetic pesticides over the years have led to their accumulations in the environment leading to poisoning as well as chronic illnesses. Their long half-lives and eco-toxicological properties have led to the adoption of natural herb based pesticides (1). Inspite of the favourable attributes of low human toxicity, reduced environment impact and rapid degradation, biopesticides have still not achieved a prominent place amongst the commercial pesticides (2). Major factors responsible for this, are the rapid degradation of the active ingredients under environmental conditions, decreasing their shelf lives. Therefore, there is an urgent need of an alternative strategy such as a nano-delivery system (3). Encapsulation of active functional ingredients in nanocarriers facilitates an inter-disciplinary approach. The study incorporates the use of an omnipotent plant known as Neem, Botanical name: *Azadirachta indica*, which is a plant with agro-medicinal properties. The presence of multiple functional ingredients in all parts of this plant including leaves, seeds, barks and flowers provides immense benefits as a biopesticide as well as a therapeutic agent (4). The most significant insecticidal neem constituent is azadirachtin, which has been established to be a prominent insecticidal ingredient and is capable of replacing synthetic pesticides (5). This plant is also known for its therapeutic capabilities specifically, a protein known as Neem Leaf Glycoprotein (NLGP). This glycoprotein can be extracted from the neem leaves and have been studied extensively in the part for its immunomodulatory properties (6). This study hypothesises that guar gum which is a galactomannan derived from the endosperm of the guar beans based biodegradable nano-carrier based encapsulation of NLGP will improve the specificity, thus facilitating targeted anti-cancer therapeutics. Further, the encapsulation of neem active ingredients from its leaves will facilitate in providing a stable biopesticidal formulation. Synthesis of guar nanoparticles (NPs) was performed using nanoprecipitation technique where the guar gum was depolymerised and encapsulated with NLGP to prepare nanocapsules. Characterisation of the NPs was performed using Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM), Dynamic Light Scattering (DLS), which confirmed the spherical morphology of the NPs which were within the size range of 30-40nm and 50-60nm for void and NLGP encapsulated NPs respectively. Further, the Differential colorimetric analysis (DSC) indicated the stability of NPs at a

temperature range of 50-60°C which was optimum since the physiological temperature is 37°C. Thermogravimetric (TGA) analysis indicated high decomposition temperature of NPs upto 350°C. Additionally, Fourier Transform Infrared spectroscopy (FTIR) and the SDS-PAGE of the nanoparticles confirmed the successful encapsulation of NLGP in the nanoformulations. Release study performed at variable temperature and pH conditions exhibited sustained and controlled release kinetics of NLGP over a period of 120h. Cytotoxicity evaluation of the NPs was performed in colon cancer cells (caco-2) cells using trypan blue exclusion assay, clonogenic assay, 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) MTT assay. Human intestinal epithelial cells (FHs) cells were also used to detect the cytotoxicity of the void and NLGP encapsulated NPs. IC50 for NLGP in FHs 74 cells was ~2 fold higher than in caco-2 cells, than in caco-2 cells suggesting that the effect of NLGP encapsulated NPs was not as significant as that in caco-2 cells. Void NPs did not present any cytotoxicity in caco-2 and FHs cells. 3D tumor spheroid assay was also performed which indicated reduction and subsequent dissociation of tumor spheroids 96h post NLGP encapsulated NPs treatment. Immunoconfocal analysis confirmed the time dependent internalisation of the NLGP encapsulated NPs by 8h. Future studies, will be focused towards employing targeted nanoformulations through the functionalisation of prepared guar gum NPs with locked nucleic acid (LNA) modified EpCAM and Nucleolin aptamers to specifically target the tumor site.





Keywords: *Azadirachta indica*, biopesticide, azadirachtin, guar gum, nanoparticle, NLGP, cytotoxicity

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Short duration iontophoresis for targeted topical peptide delivery into the skin

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***Background*:** XEP™-018 (XEP) is a highly potent 22-residue conopeptide (MW 2376 Da) found in marine snail venom (*Conus consors*) with myorelaxant and analgesic properties. In addition to its potential pharmaceutical applications, it has also been used in aesthetic dermatology to smooth the appearance of wrinkles. Given its physicochemical properties, XEP is a challenging candidate for topical passive delivery into skin. The aim of this study was to evaluate topical iontophoresis of XEP in order to determine whether it could be used to enhance its skin deposition.

***Methods*:** *In vitro* iontophoretic transport studies were conducted using porcine ear skin and vertical Franz-type diffusion cells. The effects of current density (0.1, 0.3 and 0.5 mA·cm-2), duration of current application (15, 30 and 60 min) and XEP concentration (0.1 and 1 mM in 20 mM HEPES, pH 5.6) were investigated. Passive delivery of XEP – same set-up but no current – served as the control.

***Results*:** The first study was performed using 1 mM XEP; control experiments confirmed that skin deposition of XEP following passive application was only observed after 60 min. Iontophoresis produced quantifiable skin deposition of XEP from the earliest time-point (15 min) that increased with current density. For example, after 60 min of iontophoresis at 0.1, 0.3 and 0.5 mA·cm-2, it was 3.5-, 7.9- and 13.6-fold greater than the control. The second study showed that iontophoresis for only 15 min using
0.1 mM XEP (i.e., 4-fold shorter time and 10-fold lower concentration) resulted in XEP deposition equivalent to the passive “60 min, 1 mM XEP” control. No transdermal permeation was observed.

***Conclusion*s:** The results demonstrated the feasibility of using short duration iontophoresis to improve the targeted delivery of XEP into the skin.
Given its high potency, delivery of even very small quantities of XEP is sufficient to achieve the local concentrations required in order to elicit the desired effect. Studies are now underway to optimize the formulation and delivery kinetics.

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An *in vitro* digestion – *in vivo* absorption model to examine the impact of polymers on drug absorption.

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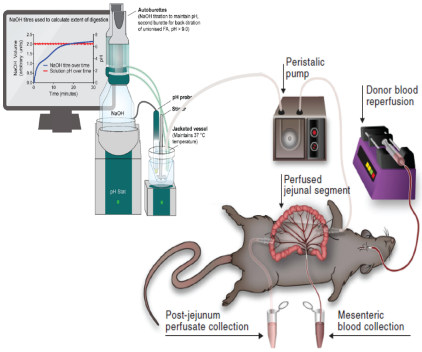
The quest for the development of ever more potent drugs has been at the expense of drugs with reasonable aqueous solubility. These poorly-water soluble drugs (PWSDs) have low and variable oral bioavailability, limiting the utility of oral administration. Lipid-based formulations (LBFs) have emerged as a promising formulation strategy to overcome the issue of solubility-limited absorption, thereby improving the oral bioavailability of PWSD. After oral dosing, a cascade of events in the gastro-intestinal (GI) tract alters the solubilizing capacity of LBF, often resulting in supersaturation and the potential for drug precipitation. To stabilize the transient, metastable supersaturated state, polymers may be added to LBF to inhibit drug precipitation, potentially resulting in increased intestinal drug absorption. To probe the beneficial effect of polymeric precipitation inhibitors (PPI) on drug absorption, a coupled *in vitro* digestion - isolated rat jejunum model,[1] has been employed here to evaluate in real time the impact of PPI on drug flux.

The results suggest that even small increases in prolongation of *in vitro* supersaturation by PPI can improve drug flux across the intestinal wall. Future studies will evaluate the impact of polymers of different structure on in vitro supersaturation and in vivo drug absorption in an attempt to achieve superior *in vivo* drug delivery.



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Encapsulation of therapeutic peptides and proteins into bulk and Cubosomes lipid cubic phase

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Therapeutic peptides and proteins represent the fastest growing class of new drugs with application as therapeutics, diagnostics and in vaccines. These complex, fragile macromolecules are, however, associated with significant drawbacks including long-term stability and degradation by the human immune system. In addition, many of these therapeutics have significant hydrophobic character, which makes encapsulation and storage an issue. Encapsulation of these therapeutics in a carrier particle can both protect the protein against degradation, deliver the protein to the desired site of action, and offer controlled release properties. Lipid based particles offer a range of advantages including potentially retaining the protein activity, and controlled release. Cubosomes are sub-micron sized (approx. 100-200nm) dispersed lipid cubic phase particles that have been shown to successfully encapsulate hydrophobic proteins and peptides in the lipid bilayer. Uptake of Cubosomes into a cellular environment has been found to be a slow process, with diffusion of therapeutic drugs across the cellular membrane over a prolonged timescale. We have formulated Cubosomes based on a mix of lipids to more successfully mimic the complexity of the native cell membrane. Peptide and protein uptake into these nanoparticles was shown to depend on the lipid composition, the cubic nanostructure, and the geometrical and charge characteristics of the encapsulated proteins. Uptake of these protein-loaded Cubosomes into a range of cell-lines was monitored using confocal microscopy.

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Aptamers as therapeutic modalities for the treatment of breast cancer brain metastases

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Metastatic brain tumours occur in approximately 25% of all cancer patients following primary malignancy treatment. Survival rates for patients with brain metastases have increased only slightly in the last twenty years. Protected by the body's most formidable barrier, the blood brain barrier (BBB), treatment options for patients is limited and thus prognosis is poor, with survival time measured in months. A promising new approach toward by-passing this barrier, is to hijack active transport mechanisms present on the endothelial cell membranes, in particular the transferrin receptor (TfR). Nucleic acid based aptamers, also known as chemical antibodies, are ideal for this purpose given the ability to generate them against a vast range of targets, their stability, and safety profile. This study has generated a bi-functional aptamer that binds to two different targets, the transferrin receptor on the blood brain barrier and EpCAM, a marker of metastatic cancer cells, which can transcytose the BBB by targeting the TfR and specifically deliver a cytotoxic payload to the EpCAM positive tumours, thus minimising cytotoxicity to healthy brain cells. To achieve this, a 14-mer DNA aptamer against the TfR was joined to a 19-mer DNA aptamer targeting EpCAM, a glycoprotein overexpressed in a number of solid tumours, including breast cancer, with a high incidence of metastasing to the brain. The specificity and selectivity of these aptamers was confirmed against a number of cells lines expressing the TfR, EpCAM or neither protein, using flow cytometry and confocal microscopy. The ability of this aptamer to enter the brain in a living system was also demonstrated using an in vivo mouse model, with the results showing specific transcytosis into the brain within 10 minutes following tail vein injection. Intercalation of the common chemotherapeutic doxorubicin showed no influence on aptamer specificity, with the conjugate specifically internalised within the targeted cells following one hour incubation. The results of this study confirm initial proof of concept that aptamers can be effective as therapeutic modalities for the treatment of neurological disorders and demonstrate the great potential this bi-functional aptamer doxorubicin conjugate has for the specific treatment of brain metastases, which will improve patient survival and quality of life, in addition to the possibility of mitigating the neurotoxic effects on healthy brain tissue.

Novel short-chain quinones against mitochondrial dysfunction induced seizures

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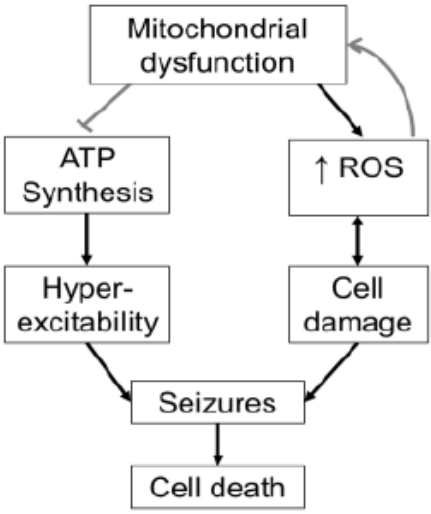
Introduction. Seizures in nearly 30% of patients are hard to control. Recent studies suggest that seizures can be treated by targeting mitochondrial dysfunction.

Aims. To generate compounds that directly target mitochondrial function and that can be developed into effective anti-seizure drugs using a rational medicinal chemistry approach.



Methods. Novel compounds were synthesized and characterized *in vitro*as a selection process before progressingwiththe most promising compounds to an *in vivo*zebrafish model of drug induced seizures as a second stage.

Results. We synthesized and characterized close to 130 novel compounds. We have improved compounds based on their ability to restore cellular viability at micromolar concentrations in the presence of a

mitochondrial inhibitor. We already identified several new compounds that show significantly better cytoprotection and lower toxicity compared to a closely-related, clinically used short-chain quinone, idebenone. Our data indicate that a specific level of solubility (logP between1.5-4.5) witha defined balance between polarity and fattiness of the side chain is essential for the compound's cytoprotective effect.

Discussion. We have identified first structure activity relationships. Upon testing enantiomer pairs of some compounds, our data suggeststhat cytoprotection is based on solubility but could also involve receptor level interactions.Now we are in a position to progressthe most promising compounds to a zebrafish model of drug-induced seizures in order to identify their efficacy to treat epilepsy by restoring mitochondrial function.

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Original vs. Generic Fixed-dose Glibenclamide/Metformin: Cost-effectiveness and Safety

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Generic drugs provide cheaper alternative and are proven to be equal to originator through bioequivalence study. However data on its cost-effectiveness were limited. We aim to assess the cost-effectiveness and safety between original and generic fixed-dose glibenclamide/metformin after changing from gliclazide plus metformin. A prospective randomized cross-over study among type 2 diabetes patients treated with stable dose of at least 240mg of gliclazide plus 1000mg of metformin was conducted in the Out-Patient Department. They were randomly divided into two groups to receive either original (Group A) or generic (Group B) glibenclamide/ metformin 2.5/500 mg two tablets twice a day for 12 weeks. After 12 weeks, patients received original drug were switched to generic whereas patients that received generic drug were switched to original for another 12 weeks. Baseline glycosylated haemoglobin (HbA1c), number of hypoglycaemic episodes, adverse effects and cost of drug acquisition were measured and repeated at 12-week and 24-week. Incremental cost-effectiveness ratio (ICER) was calculated. Eighty four patients with mean age of 58.01 years and diabetes duration of 12.84 years participated. Baseline characteristics were similar between the groups. Mean HbA1c reduced significantly (-0.30%, $p<0.01$) after changing from gliclazide plus metformin to fixed-dose glibenclamide/metformin regardless of original or generic. However, more patients achieved target HbA1c ≤6.5% with original compared to generic (10.71% vs. 1.19%, $p<0.01$). The ICER showed additional USD 69.28 needed to treat patients to target with generic glibenclamide/metformin compared to original. There was no significant difference in number of hypoglycaemic episodes after switched to fixed-dose glibenclamide/ metformin. However, significantly less hypoglycaemia episodes occurred when changed from generic to original glibenclamide/metformin in Group B. Six patients (7.14%) experienced drowsiness and unable to perform activity with generic fixed dose glibenclamide/metformin but not with the original. Original glibenclamide/metformin was more cost-effective and well tolerated albeit generic was equally effective as original in lowering HbA1c.

The role of gelsolin in 5-Fluorouracil resistance in colorectal cancer: molecular mechanisms and potential therapeutic implications

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Colorectal cancer (CRC)is a global health concern and is one of mostcommon types of cancer1. Current first line chemotherapeutic treatmentof CRC comprises 5-Fluorouracil (5-FU)-based regimen2.However, response to 5-FU-based therapy varies in patients, and development of drug resistance also impedes the effectiveness of treatment. Therefore, there is a pressing need to understandthe mechanisms of drug resistance and identify new targets to help overcome chemoresistance.Herein, we identifythat gelsolin, an actin-binding protein, can contributeto resistance to 5-FU inCRC cells. Using CRCcell lines, we showed that gelsolin protectedCRCcells from 5-FU-induced cell death, while silencing gelsolin sensitized cells to 5-FU treatment. Furthermore, gelsolin modulatedautophagy, an important cell survival process, as a cytoprotective mechanism in response to 5-FU. Mechanistically, gelsolin regulatedthe expression and activities of lysosomalenzymes, including cathepsin B, D, and L, which are involved in autophagy. We also demonstratethat gelsolin could be a potential prognostic marker for CRCpatients, where patients with high levels of gelsolin expression showed shorter disease-free survival, compared to those with low levels of expression.Moreover, high expression of both gelsolin and cathepsinscorrelatedwith poorer survival of CRC patients. In summary, we demonstrated a role of gelsolin in regulating autophagy via modulating lysosomal enzymes, which contributes to 5-FU resistance in CRC. Our findings highlight gelsolin as a potential target for combatingresistance to 5-FU based chemotherapy, and as a possibleprognostic factor in patient survival of CRC.

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X-ray Crystallography and Paramagnetic NMR to Expedite Structure-Based Drug Discovery

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Obtaining structural information during ligand binding is useful to understand SAR and progress SBDD. While X-ray methods deliver unrivalled structural details, NMR methods are useful from the early detection of weakly binding hits to chemical shift (CS) based docking of a bound ligand complex. Any ligand-induced conformational changes often frustrate the analysis reducing the capability of the CS.

By attaching a rigid lanthanide ion to the protein through bioconjugation of our synthetic lanthanide binding tags[1] (LBT), we can enhance the CS parameter by virtue of the paramagnetic *pseudo contact shift* (PCS). This delivers long-range distance and angular restraints (up to 50Å from the bound ion) that positions each nuclei (protein or ligand) within a metal-centred three-dimensional coordinate system via its PCS. Structural information is derived from simple and sensitive spectra, applicable to quite large proteins far beyond the reach of the usual short range NOESY data that requires the difficult assignment of side-chain atoms.

We exemplify the combined X-ray and LBT approach to unravel large (~20Å) ligand-induced conformational changes revealing an unexpected cryptic binding pocket that reconciles species-selective SAR during the evolution of sub-micromolar inhibitors of HPPK, an antimicrobial target from the folate biosynthesis pathway[2].

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Design & Development of Novel Selective Sialyltransferase Inhibitors using Computational Tools

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Hypersialylation of tumor cell surface proteins along with a upregulation of sialyltransferase (ST) activity is a well-established hallmark of cancer. Due to the critical role of STs in tumor growth and progression, ST inhibition has emerged as a new antimetastatic strategy for a range of cancers. Human STs are divided into subtypes based on their linkage and acceptor molecule, with each subtype controlling the synthesis of specific sialylated structures with unique biological roles. This has important implications for inhibitor development, as STs also play significant roles in immune responses, inflammation, viral infection, and neurological disorders. Thus, the current goal to advance to the clinic is to develop subtype selective, cell-permeable and synthetically accessible, small-molecule ST inhibitors.[1]

The recent reports of mammalian ST crystal structures enables structure-based design for the first time. Of these, three enzymes were described, representing the main STs subfamilies: ST3Gal I, ST6Gal I and ST8Sia III.[2]

The purpose of this study is to design and prepare new inhibitors using efficient and non-expensive synthetic methods. Thus, we prepared a series of 20+ nucleoside derivatives by modifying key fragments based on our SAR model. Docking and molecular dynamics were used to refine our design approach. The preliminary assessment of their cytotoxicity reveals very low toxic profiles, and their complete biological evaluation is currently in progress.

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Microwaves and gold-nanoparticles based protein therapy

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The effects of electromagnetic radiation on proteins has become of interest as the electromagnetic sources such as mobile phones, transmission towers and power lines increased. Further more the ability to control the dynamics of proteins using microwave radiation can be harness for therapeutics application. In this work a method that relies on molecular dynamics and non-equilibrium molecular dynamics is presented to understand the dynamics of proteins/enzymes under low and low and high intensity microwave fields of different frequencies is presented. The method is applied for two proteins namely FLT3 (a protein coding gene) and Lactate dehydrogenase. The possibility of using gold nano-particles to further module the dynamics of proteins for therapeutics will be explored.