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The small heat shock protein (sHSP) HSP27 is an essential, redox-sensing molecular chaperone that is ubiquitously expressed in humans. Expression of HSP27 is upregulated under conditions of cellular stress, upon which HSP27 acts to prevent protein misfolding, maintain redox balance, and uphold cytoskeletal integrity. Detailed structural investigations of the structure and function of HSP27 have proved challenging due to its formation of dynamic, polydisperse oligomers ranging from 40 to 1000 kDa in mass. Here, we have used a combination of solution-state nuclear magnetic resonance (NMR) spectroscopy and native mass spectrometry (MS) to provide structural and dynamical insight into the tertiary and quaternary structure of HSP27 under reducing and oxidizing conditions. We quantified stoichiometries within the polydisperse ensemble of HSP27 oligomers using native MS, and demonstrated redox-dependent formation of monomeric HSP27. To study redox-induced structural effects at atomic resolution, we utilized the excised alpha-crystallin core domain of HSP27 (cHSP27; 10 kDa), which forms both covalent and non-covalent dimers (20 kDa). cHSP27 was analyzed with $^{15}$N spin relaxation experiments to quantify the motions of its backbone on both fast (pico-nanoseconds) and slow (micro-milliseconds) timescales. Fast motions were independent of redox state; however, under reducing conditions, the non-covalent dimer of cHSP27 exchanges with a transiently populated state (1.5%) that has a lifetime of ~600 microseconds. Large-scale structural rearrangements occur upon formation of this energetically excited state, which is also formed under acidic conditions. Exchange between the non-covalent dimer and this minor state was found to be concentration-dependent, indicating that the excited state corresponds to monomeric cHSP27. Under oxidizing conditions, the chaperone activity of HSP27 against multiple protein substrates was diminished, indicating that monomer-dimer exchange within the full-length protein is essential for its proteostatic function.
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Memory CD4 T cells are the primary target of HIV/SIV infection, are massively depleted in acute infection and partially rebound with the onset of the adaptive immune response. Follicular helper T cells (TFH) in lymph nodes provide B cell help and are critical to a robust humoral immune response, and are a major compartment of HIV infection and replication in chronic infection. Here, we trace the infection of memory CD4 T cells from early infection using DNA and RNA qPCR to quantify viral replication in TFH and other T cell subsets, and use RNA probes in flow cytometry to examine the phenotype of individually infected cells. Rhesus macaques were infected with SIVmac251 and serially sampled to measure SIV in blood, lymph nodes, and spleen. At all timepoints, copies of spliced and unspliced SIV RNA were highest in CD4dim cells, due to Nef down-regulation of surface CD4 during active infection. TFH increased as a proportion of all CD4 memory T cells throughout infection, and both SIV gag DNA, gag RNA, and tat/rev RNA were highest in TFH. In early infection, memory CD4 T cell subsets contributed equally to SIV DNA and RNA, but by six months post infection, TFH make up the bulk of SIV infected and virus producing cells. This study maps the dynamics of SIV in the earliest stages of infection across multiple tissues to track the compartments of CD4 T cells that are responsible for virus replication and infection.
The amyloid motif of protein quaternary structure has recently been linked to many diseases of ageing, including diabetes (insulin), Parkinson’s (α-synuclein) and Alzheimer’s (amyloid-β, or Aβ). This supramolecular organizational scheme also emerges in functional contexts such as the synthesis of melanin in humans (Pmel12) and the formation of biofilms in bacteria (FapC). Experimentally, the formation of these fibrillar species demonstrate a nucleation-elongation polymerization mechanism, in which the rate-determining association of few protein monomers into a growth-competent nucleus is followed by fast incorporation of further soluble subunits into insoluble fibers. The concomitant large ranges of timescales and length scales of these reactions pose a challenge to simulating the entire reaction landscape computationally, but one solution is projecting the whole dynamics onto fewer degrees of freedom in order to make long simulations feasible. In this work, we describe such a coarse-graining scheme in which we construct Aβ17-42 monomers from diatomic amino acids interacting through a simple intermolecular potential dictated by the fibril structure solved with NMR and amide H/D exchange. By limiting the number of atoms involved we are able to probe, effectively, millisecond timescales. We show that such a simple, structure-based model is enough to capture kinetic and thermodynamic parameters describing the elongation chain-reactions of amyloidogenesis that are quantitatively similar or identical to experimental values, as well as extracting experimentally inaccessible values. These results imply that the instructions for the fibrillation mechanism are at least partially encoded in just the final structure of the aggregates, thereby providing an example of the structure-function paradigm of protein science and linking the observed ubiqutities of amyloid structure and reaction mechanisms demonstrated by many organisms and cell lines.
Prostate cancer is the most common malignancy and second leading cause of cancer deaths in American men, with approximately 220,800 diagnoses and 27,540 deaths in 2015. The five-year survival for local disease is nearly 100%, compared to only 28% for metastatic disease. This outcome disparity frames the major clinical challenge associated with PCa: distinguishing those men who are likely to get metastatic disease, which may be prevented by specific and early therapy, while minimizing the iatrogenic morbidity associated with overtreatment of indolent disease. Consequently, a great deal of PCa research is focused on finding molecular and genetic biomarkers that facilitate early and accurate identification of men with potentially high-risk tumors. To identify clinically important molecular subtypes of prostate cancer (PCa), we characterized the somatic landscape of aggressive tumors using deep whole genome sequencing. In our discovery set of 10 tumor/normal pairs with Gleason scores of 8-10 at diagnosis, coordinated analysis of germline and somatic variants, including single nucleotide variants, indels, and structural variants, revealed biallelic BRCA2 disruptions in a subset of samples. Compared to the other samples, the PCa BRCA2-deficient tumors exhibited a complex and highly specific mutation signature, featuring a 2.88-fold increased somatic mutation rate, depletion of context-specific C>T substitutions, and an enrichment for deletions, especially those longer than 10-bp. We next performed a BRCA2 deficiency-targeted reanalysis of 150 metastatic PCa tumors, and each of the 18 BRCA2-mutated samples recapitulated the BRCA2 deficiency-associated mutation signature, underscoring the potent influence of these lesions on somatic mutagenesis and tumor evolution. Among all individuals with BRCA2-deficient tumors, only about half of 21 carried deleterious germline alleles. Importantly, the somatic mutation signature in tumors with one germline and one somatic risk allele was indistinguishable from those with purely somatic mutations. Further, any test designed to leverage BRCA2 status as a biomarker for PCa must consider both germline and somatic mutations, and all types of deleterious mutations. Our observations clearly demonstrate that BRCA2-disrupted tumors represent a unique and clinically relevant molecular subtype of aggressive PCa, highlighting both the promise and utility of this mutation signature as a prognostic and treatment-selection biomarker.
Abstract Title

Colitis susceptibility in p47phox-/ mice is mediated by the microbiome

Abstract Authors

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Background: Chronic granulomatous disease (CGD) is caused by defects in nicotinamide adenine dinucleotide phosphate oxidase 2 (NOX2) complex subunits (gp91phox (a.k.a. Nox2), p47phox, p67phox, p22phox, p40phox) leading to reduced phagocyte-derived reactive oxygen species production. Almost half of patients with CGD develop inflammatory bowel disease, and the involvement of the intestinal microbiome in relation to this predisposing immunodeficiency has not been explored.

Results: Although CGD mice do not spontaneously develop colitis, we demonstrate that p47phox-/ mice have increased susceptibility to dextran sodium sulfate colitis in association with a distinct colonic transcript and microbiome signature. Neither restoring NOX2 reactive oxygen species production nor normalizing the microbiome using cohoused adult p47phox/- with B6Tac (wild type) mice reversed this phenotype. However, breeding p47phox/- mice and standardizing the microflora between littermate p47phox/- and B6Tac mice from birth significantly reduced dextran sodium sulfate colitis susceptibility in p47phox/- mice. We found similarly decreased colitis susceptibility in littermate p47phox/- and B6Tac treated with Citrobacter rodentium.

Conclusions: Our findings suggest that the microbiome signature established at birth may play a bigger role than phagocyte-derived reactive oxygen species in mediating colitis susceptibility in CGD mice. These data further support bacteria-related disease in CGD colitis.
Non-replicating viral vectored vaccines have shown a remarkable capacity to induce systemic CD8^+ T-cell responses in animals and humans. To date, the greatest immunogenicity has been obtained through a heterologous prime boost regimen, where vaccination with an Adenoviral vector is followed 8 weeks later by a Modified Vaccinia Ankara virus (MVA) boost. However, protection against liver-stage malaria relies on the induction of unusually high numbers of circulating CD8^+ T-cells, which are required at the time of infection in order to find and kill infected hepatocytes.

Utilizing novel vaccine strategies, antigen specific CD8^+ T-cells were targeted to the liver, resulting in a ten-fold increase in liver resident (CD69^+ CXCR6^+) CD8^+ T-cells. This resulted in 100% sterile protection when C57BL/6 mice were challenged with transgenic OVA expressing *P.berghei* sporozoites, an efficacy level greatly exceeding conventional vectored immunisation. Importantly, protection appeared well-maintained, with sterilizing responses observed for at least two months post vaccination. Furthermore, this regimen showed to be protective against the clinically relevant malaria antigens, PfLSA1 and PfTRAP in BALB/c and CD1 mice using a transgenic parasite challenge. By generating long-lived antigen specific CD8^+ T-cells resident in the liver, this new vaccination approach provides a substantial advance in clinically relevant malaria vaccine development.
Abstract Title | No cell left behind: Residual ovarian spheroids drive recurrence and are sensitive to the pro-oxidant elesclomol

Abstract Authors | Ian S. Goldlust, Kelli Wilson, Ludmila Szabova, Xiaohu Zhang, Lesley Mathews-Griner, Maria Vias, Anna Piskorz, Rory Stark, Lee Mendil, Monica Kasbekar, John Braisted, Rajarshi Guha, Crystal McKnight, Paul Shinn, Donna Michelle-Smith, Zoe Weaver Ohler, Mindy Davis, Udo Rudloff, Sam Michael, Madhu Lal-Nag, Scott Martin, Christina Annunziata, Marc Ferrer, James D. Brenton, Craig Thomas.

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Sphere forming cells persist in the ascitic fluid of patients with high-grade serous ovarian cancer after first-line therapy and likely contribute to relapse and metastasis. These residual tumor spheres, which are enriched for cancer cells by up to 95% based on detectable TP53 mutations, are slow growing, resistant to platinum-based chemotherapy, and remain a large obstacle towards durable remission. Screening established, rapidly dividing monolayer cell lines in proliferation assays has failed to produce chemotherapeutics capable of eradicating this slow growing population. To identify a consolidation therapy that targets these residual tumor cells we screened nearly 2000 mechanistically annotated, approved and investigational drugs in three cell lines (PEO1, PANC1, A375M) cultured in spheroid and conventional monolayer. To elucidate targetable genes and pathways responsible for spheroid maintenance we performed a whole-genome RNAi screen against PEO1 in both culture conditions. Our pharmacological and genetic profiling provided mechanistic insight into the baseline changes that occur when cells are grown in three dimensional, anchorage independent conditions and identified susceptibilities specific to spheroid populations. Consistent with residual disease, cultured spheres were resistant to many common chemotherapeutics, most notably proteasome inhibitors, but were highly sensitive to the pro-oxidant elesclomol. We verified elesclomol’s activity in ex vivo cancer spheroids extracted from the ascitic fluid of patients with advanced high-grade serous ovarian cancer using a simple, culture-free technique. Expression profiling of cultured and ex vivo spheres revealed broad downregulation of genes involved in cell cycle signaling (AURKA, AURKB, PLK1, CDK1, CCNA2, CCNB1, CCNB2, GMNN, CHEK1). Treatment with elesclomol induced expression of chaperone proteins (HSP6, HSP7, HSPA1A, HSPA1B, DNAJA4, DNAJB1), metallothionein proteins (MT1F, MT1M, MT1P2, MT1X, MT2A), and genes involved with the oxidative stress response (HMOX1, ABCB1, SLC7A11) consistent with increased reactive oxygen species caused by high intracellular Cu2+. To pursue this drug as a consolidation therapy in vivo, we evaluated elesclomol’s toxicity, pharmacokinetic properties, and efficacy in a murine model for recurrent high-grade serous ovarian cancer. By targeting residual tumor cells with elesclomol after successful treatment with platinum-based therapeutics we hope to prevent recurrence.
Chemotherapy is often administered in group settings, which allows for social influence between patients. Patients can influence one another through contact or solely based on co-presence. Social influence in turn can affect health directly as well as indirectly mediated by stress response. This influence is likely strongest when patients are familiar with one another; here, we define familiarity as patients being consistently co-present (CCP) over the course of their chemotherapy cycles. We provide empirical results to support the hypothesis that patients who are CCP with one another influence one another’s health in the chemotherapy ward. We use data on the population of all 4,691 out-patients in a single chemotherapy ward in Oxfordshire, UK from Jan 1, 2000 to Jan 1, 2009. We assume that it is possible for patients to influence one another only if they are CCP more than expected by chance, adjusting for chemotherapy schedules. We model 5-year survival following chemotherapy to examine social influence with an indicator for whether one is CCP with at least one other patient (alter). We also create count variables for the number of CCP alters who survive or die in the 5 years following chemotherapy. We adjust for age, sex, cancer severity, total person-hours of co-presence, and chemotherapy duration. We find that patients CCP with at least one alter (n=2,704) have their odds of 5-year survival increased by a factor of 1.59 (95% CI: 1.36, 1.85). Additionally, every CCP alter that survives for 5 years following chemotherapy increases one’s own odds of survival by a factor of 1.13 (95% CI: 1.08,1.19), and every CCP patient that dies within 5 years decreases one’s odds of survival by a factor of 0.93 (95% CI: 0.89,0.96). These results indicate that social influence on health in the chemotherapy ward is a joint function of being CCP with other patients, as well as their health status. Given the presence of both positive and negative influence effects, ethically maximizing outcomes based on these results may not be straightforward and require further research. It is also important to note that we observe these effects based solely on co-location data, which are relatively easy and cheap to obtain, but are often considered inferior to relational data for detecting social influence. Our results suggest that data of this nature may be used to study the presence of social influence in settings where traditionally higher-quality data could not be obtained.
**Abstract Title**

Novel actin-binding proteins with multifunctional properties

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Actin filaments are traditionally labelled with antibodies, phalloidin, or directly functionalized to bind dyes or biotin. We have developed a new approach, which uses Adhirons, small (~12kDa), non-antibody proteins that have specific and tight binding to the protein of interest (Tiede et al., 2014). These proteins are raised with phage display assay and have the high thermostability phytocystatin consensus core. The advantage of using Adhirons is that these proteins are small, and once isolated by screening, can be expressed and purified using *E. coli*, or expressed as GFP-tagged constructs in mammalian cells for functional studies. A single cysteine can also be introduced at the C-terminus to allow their functionalization.

We isolated four actin binding Adhirons (Adh2, 6, 14 and 24) by phage display assay. Measurements of their binding affinity to F-actin, using actin spin-down assays, showed a tight binding (the dissociation constant was less than 0.5 μM) for three of the Adhirons (Adh6, 14 and 24). These three Adhirons seem to bind to distinct binding sites on F-actin, however myosin-5 subfragment 1 (S1) competes all of them off F-actin. They also inhibit myosin-5 actin-activated ATPase activity, which might be because tightly-bound Adhiron blocks the weak initial binding of S1,ADP,Pi to F-actin, but it is also possible that reduced binding sites available on F-actin due to actin bundling by Adhirons. GFP-tagged versions of Adh6, 14 and 24 stain actin, when expressed in live cells. Adh14 localizes to filaments, whereas Adh24 stains focal adhesions. Adh6 seems to label both filaments and vesicular structures. Adhirons 14 and 24 were additionally tested for their ability to label actin filaments in cells, using an Adhiron-Cys, which was biotinylated, and visualised with fluorescent streptavidin. Confocal microscope images showed that Adh14 was localized to filaments similarly to phalloidin, and Adh24 additionally labelled some vesicular structures.

As these proteins are small, easy to express and are already proven to bind to actin effectively, they are likely to be useful as a new approach to label actin, not only in fluorescence microscopy, but in *in vitro* assays, such as attaching actin filaments to glass slides for *in vitro* motility assays, or to plastic beads for optical trapping assays.

**Reference:**

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<th>An Allometric Analysis of Sex and Sex Chromosome Dosage Effects on Subcortical Anatomy in Humans</th>
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Structural neuroimaging of humans with typical and atypical sex-chromosome complements has established the marked effect of both sex and X-/Y-chromosome dosage on total brain volume (TBV) and identified potential cortical substrates for the psychiatric phenotypes associated with sex-chromosome aneuploidy (SCA). In a cohort of 354 humans with varying karyotypes (XX, XY, XXX, XXY, XYY, XXXY, XXXXY), we investigate sex and SCA effects on subcortical size and shape—focusing on the striatum, pallidum and thalamus. We find large effect-size differences in the volume and shape of all three structures as a function of sex and SCA. We correct for TBV effects with a novel allometric method harnessing normative scaling rules for subcortical size and shape in humans, which we derive here for the first time. We show that all three subcortical volumes scale sub-linearly with TBV amongst healthy humans - mirroring known relationships between subcortical volume and TBV amongst species. Traditional TBV correction methods assume linear scaling and can therefore invert or exaggerate sex and SCA effects on subcortical anatomy. Allometric analysis restricts sex differences to (i) greater pallidal volume (PV) in males, and (ii) relative caudate head expansion and ventral striatum contraction in females. Allometric analysis of SCA reveals that supernumerary X- and Y-chromosomes both cause disproportionate reductions in PV, and coordinated deformations of striatothalamic shape. Our study provides a novel understanding of sex and sex-chromosome dosage effects on subcortical organization, using an allometric approach that can be generalized to other basic and clinical structural neuroimaging settings.
A fundamental axiom in human neuroimaging has formed from the large body of work showing inter-individual differences in cortical brain structure, as measured by magnetic resonance imaging (MRI). Moreover, recent research suggests that these inter-individual differences in the structure of a given brain region co-vary with inter-individual differences in the structure of other brain regions—a finding that has been aptly termed structural covariance (SC). However, compared to studies of functional brain networks, where a brain network is derived for each subject, SC has been limited to analyses at the group-level due to the use of a single morphological feature (e.g. cortical thickness or gray matter volume) in the generation of the brain network. Although some recent work has provided novel methods to generate individual SC networks, these have still been restricted by the use of a single morphological feature, and have not made use of other aspects of brain morphology, such as regional brain curvature, surface area, and cortical white matter content. Thus, using a multi-parametric MRI sequence, we extracted 10 morphological brain features for each brain region in each subject, and for each region we computed its correlation (Pearson’s $r$) with each of the remaining brain regions, resulting in a single morphological connectivity profile for each subject. Neuroimaging data was taken from the Neuroscience in Psychiatry Network, and was comprised of 300 adolescents (aged 14-25, 150 male), arranged into a series of 5 bins of equal size and equal sex distribution. Graph theoretic analyses of the individual morphological networks showed high clustering and short mean path lengths relative to random networks, yielding a high small-world coefficient typical of brain networks. Additionally, across subjects, both the average and rate of nodal morphological connectivity was found to be correlated with the rate of cortical thinning and the rate of cortical myelination, demonstrating a relationship between the development of brain morphology and the change in brain connectivity during adolescence. Finally, we generated a gene co-expression network using the freely available microdissection data from the Allen Institute for Brain Science, and found a significant correlation ($r = 0.45$) between the edges of this gene co-expression network and the edges of the mean individual brain morphological network, providing evidence for biological underpinnings of this method of structural brain connectivity.
POSTER ABSTRACTS
The removal of damaged mitochondria through autophagy machinery, or mitophagy, is a key aspect of proper cell health and is one of the ways our cells prevent us from developing Parkinson’s disease. Mitophagy is initiated by the PINK1/Parkin pathway, which detects mitochondrial membrane depolarization or the presence of an abundance of misfolded proteins within a mitochondrion. Once marked by Parkin, the damaged mitochondrion is degraded. Recent studies suggest that Parkin activity can also identify damage at a specific spot of an otherwise healthy mitochondrion. Acting as a first response mechanism, Parkin may be able to initiate a form of localized mitophagy, facilitating deletion of the damaged portion of the mitochondrion without sacrificing the entire organelle. How the damaged portion of mitochondrion is removed remains unclear. Is the ER involved? Fission machinery? Vesicle formation? Using correlative light and electron microscopy (CLEM), we are addressing the ultrastructural sequence of events for localized mitophagy and determining the involvement of other cellular structures in this process.
Elucidating the mechanism of piperaquine resistance in *Plasmodium falciparum* malaria in Cambodia

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Artemisinin combination therapies (ACTs) are currently the first-line treatments for *Plasmodium falciparum* malaria worldwide. ACTs, which combine a short-acting artemisinin derivative with a long-acting antimalarial partner drug with a different mechanism of action, are designed to efficiently clear parasitemia and prevent the development of drug resistance. In some countries of Southeast Asia (SEA), the current treatment for *P. falciparum* malaria is the ACT dihydroartemisinin (DHA)-piperaquine (PPQ). Unfortunately, the emergence and spread of DHA-PPQ resistance has now been reported at multiple sites in Western Cambodia, which poses a severe risk of widespread resistance to DHA-PPQ and other ACTs. Recent fieldwork by our group identified *P. falciparum* strains that show markedly reduced susceptibility to PPQ in Cambodia. In a genome-wide association study of parasite responses to PPQ exposure *in vitro*, we discovered a single-nucleotide polymorphism (SNP) on chromosome 13 coding for an exonuclease that strongly associates with reduced PPQ susceptibility *in vitro* and DHA-PPQ failures in patients. We are currently using the CRISPR-Cas9 system to edit the wild-type and mutant exonuclease SNP into PPQ-resistant and PPQ-sensitive parasites, respectively. After transfections and successful editing events are verified, we will perform PPQ survival assays to determine whether the mutant SNP confers PPQ resistance. We are also genotyping the exonuclease SNP in contemporary parasite isolates from Cambodia and neighboring countries to monitor the spread of PPQ resistance in SEA. In addition to validating the exonuclease SNP as a molecular marker of PPQ resistance, we aim to identify the causal genetic determinant of PPQ resistance and use appropriate biochemical methods to elucidate its molecular mechanism. These studies will provide novel insights into the mechanism of PPQ resistance in *P. falciparum* and will help to monitor and prevent the further spread of PPQ resistance and DHA-PPQ failures in SEA.
Thalidomide is widely known for its teratogenic effects during embryogenesis. After an initial ban, the discovery that thalidomide had anti-inflammatory and anti-angiogenic effects restored its potential for clinical use. Thalidomide is now utilized in the treatment of multiple myeloma, ENL, Crohn's disease, macular degeneration and several types of cancers. However, the drug still retains unwanted side effects including being harmful to the developing embryo, and causing peripheral neuropathy. Moreover, the low solubility of thalidomide has prevented the development of an intravenous formulation. Oral pharmacokinetics studies have shown that thalidomide absorption from the gastrointestinal is very slow, with peak plasma concentrations not observed until 2.9-5.7 hours after administration. The side effects and solubility have catalyzed the development of new analogs, with the aim of synthesizing compounds with more potent activities and reduced toxicities. Here, we screened libraries of new thalidomide analogs for potent anti-inflammatory and anti-angiogenic effects in vitro and in vivo. Lead compounds were also screened for teratogenic side effects, and have been pushed through preclinical development in mouse models of human prostate cancer. The mechanism through which these drugs may be anti-angiogenic and teratogenic has been studied in in vitro and in vivo models of angiogenesis, and a common mechanism of teratogenicity via a loss of early developing blood vessels has been identified. Using the angiogenesis data, we performed three dimensional quantitative structure activity relationships to identify the functional groups required for activity. Development of this biocomputational model will aid in the design of more potent compounds, and combined with in vitro and in vivo screening will allow for rapid development of the drugs.
Unlike common multi-factorial diseases, the aetiology of rare monogenic disorders of severe insulin resistance can be traced to non-synonymous single nucleotide polymorphisms (nsSNPs), often affecting insulin signalling components. Similar mutations in many of the homologous proteins in *C. elegans* also have phenotypes indicative of decreased nutrient signalling, including increased dauer diapause formation and increased longevity. By using a recent *C. elegans* resource provided by the Million Mutation Project (MMP), a semi-nonbiased exploration of viable alleles throughout the worm genome without any previously recognized phenotype can be performed, similar to identifying human SNP’s in viable persons through whole genome sequencing. Partly due to its large size (33 kb gene; 1,846 amino acids), *daf-2* had 40 new MMP alleles added to the 26 previously reported *daf-2* alleles identified in large scale genetic screens. Twelve of the new MMP *daf-2* alleles had changes in conserved amino acids that in humans cause severe disorders of insulin resistance. Two backcrosses (2xBC) were performed to remove most of the background mutations and at least three independent isolates were used for phenotyping to reduce the effect of background mutations on the phenotypes. The high temperature (27.3 °C) dauer assay (HID) phenotype is a very clear indicator for weak loss-of-function alleles in *daf-2*. Using this strategy, seven new HID alleles have been identified. CRISPR-Cas9 was used to recreate and confirm three alleles with positive phenotypes and two alleles without any apparent phenotype. Additionally, one conserved allele with WT phenotype was remade using CRISPR-Cas9 along with two human alleles at that amino acid, which remarkably showed the same gradient of phenotype observed in humans. Finally, a new N-term DAF-2 polyclonal antibody was developed to test protein expression. This study highlights the importance of modelling evolutionarily conserved human disease alleles in one of the simplest organisms with this pathway.
Nanomedicine arguably has the unique potential of providing targeted multi-receptor driven drug delivery to discrete areas of the body through careful organisation and expression of proteins and other biomolecules on the surface of nanoparticles. The current issues facing nanomedicine as a drug candidate can be split into two classes: synthesis and characterisation. On the one hand being able to controllably organise biomolecules on the surface of nanoparticles in a reproducible manner has proven difficult but some progress has been made, for example through antibody and other small biomolecule functionalisation to particle surfaces in a controlled manner \([1,2]\).

On the other without adequate characterisation techniques for these new bio-nano constructs we are unable to determine the reproducibility of these syntheses beyond average values for a given batch of nanoparticles, methods such as mass spectroscopy, dynamic light scattering and agarose gel give reliable data about the whole but miss the precise particle-by-particle analysis required by modern medical standards.

Some steps have been made towards a more precise approach using monoclonal antibodies to probe for specific sites related to a biomolecules activity \([3]\) and the use of microfluidic devices to probe nanoparticle-surface interactions. But to classify the orientation and conformation of several classes of biomolecules on a nanoparticle in this way would require a large body of work and time.

Therefore a new and high-throughput technique must be developed to specifically and reliably classify biomolecules on the surface of nanoparticles. We propose the use of phage display technology as a tool to tackle this challenge.

By raising a library against a specific biomolecule through series of binding and recovery steps known as panning, one can use this to elucidate orientational and conformational information of biomolecules on particles. Phage display’s versatility in application means that libraries raised against these biomolecular characteristics can be used to select or eliminate particular traits from a batch of nanoparticles. The libraries can also be used to create selective binding motifs which can be attached to nanoparticles potentially conferring selectivity onto the nanoparticles. The direct relationship between phage DNA and expressed antigen allows for isolation, sequencing, barcoding and possible modification of the biomolecule for improved performance.

References:
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<th>Abstract Title</th>
<th>Molecular Characterization of FERM and PDZ Domain Containing 1 (Frmpd1), a Candidate Gene Necessary for Rod Photoreceptor Maturation</th>
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<tr>
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<td>Christie K. Campla, Jung-Woong Kim, Hyun-Jin Yang, Jerome Roger, Stephanie Halford, Sumathi Sekaran, and Anand Swaroop</td>
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Vision loss from retinal degeneration is largely associated with the death or dysfunction of photoreceptor cells (rods and cones). While cones are responsible for high resolution and color vision under normal daylight conditions, they are greatly outnumbered by rods, which provide night vision. The photoreceptors are highly metabolically active cells, yet rods seem to be more vulnerable to disease than cones, and often the degeneration of rods leads to a secondary loss of cone function and severe visual defects. Therapeutic approaches are targeted toward protecting against cell death or replacing photoreceptors, as no new rods or cones are formed after maturation. Thus, a thorough understanding of the processes governing proper photoreceptor development and homeostasis would aid in the design of more effective treatments for retinal degeneration.

The differentiation of rod photoreceptors is stringently regulated by a number of transcription factors, which include NRL (neural retina leucine zipper) and CRX (cone-rod homeobox). We hypothesized that genes dramatically increasing in expression during rod photoreceptor development and regulated by NRL and CRX would play a substantial role in the functional maturation of rods, and that cellular adhesion genes involved in this process would be important for establishing and stabilizing proper organization of photoreceptors within the outer nuclear layer. RNA-seq data from FACS-sorted murine rod photoreceptors (from transgenic mice expressing GFP under the control of the Nrl promoter) was used to select for candidate cell adhesion genes differentially upregulated in rods from P2-P28. This project aims to elucidate the expression (by RNA-Seq, PCR, Western immunoblotting, in situ hybridization, and immunohistochemistry), transcriptional regulation (by 5′-RACE, ChIP-seq, luciferase assay, and in vivo promoter assay) and function (knockdown mouse model assessment) of one such gene, Frmpd1.

This research should contribute to a better understanding of the architecture of the photoreceptor layer and optimization of rod function; it may prove especially useful in designing cell-based therapies for retinal disease, where the successful incorporation of transplanted photoreceptors requires proper cell organization, reestablishment of cell-cell junctions and synapse formation.
Primary immunodeficiency disorders (PIDs) are a diverse group diseases caused by genetic defects affecting immune functions. However, up to a third of patients may have novel mutations not previously associated with PID. By studying the causative mutations of PID patients, we can further elucidate the molecular mechanisms underlying immunity. Here, we describe a set of patients who presented with T cell lymphopenia, splenomegaly, lymphadenopathy, and liver disease, along with other immune problems, such as autoantibody production and colitis. Through whole exome sequencing, it was found that the patients had mutations in either GTPase of the immunity-associated proteins (GIMAP) 5 or GIMAP6. The GIMAP proteins are a family of conserved GTPases expressed in hematopoietic tissue with a poorly understood role in leukocyte development and survival. Study of cells from patients and knockout mice have shown proliferative and survival defects in T cells. Furthermore, various assays involving immunoprecipitations, microscopy, and flow analysis combined with previous data suggest that GIMAP5 and GIMAP6 may play a role in either autophagy or endosome trafficking. In studying this and other patients, we hope to further determine the immune pathways needed for normal immune function, and to use this knowledge to create novel diagnostic tools and therapies for the treatment of PIDs and other immune conditions.
Malaria parasites in Cambodia have begun to develop resistance to several first-line antimalarial drug therapies – artemisinin-based combination therapies (ACTs). Preventing the spread of drug resistant parasites through Southeast Asia and to Africa is a top priority for global malaria elimination campaigns. The ability to detect these small molecule drugs in malaria patient samples at the point-of-care would allow healthcare workers to identify previous treatment failures and adjust future treatment to improve efficacy and reduce the spread of resistant parasites. A simple assay to detect these drugs from patient samples would also allow for real-time reporting of drug use for mapping and compliance studies.

We aim to develop a low-cost, field-based test to detect several slow-clearing ACT drug compounds from fingerstick blood samples. We hope to identify drug-specific aptamers via an inverted SELEX protocol in which we immobilize a DNA library and isolate structure-switching sequences that are released upon binding drug target in solution. We are currently focusing on aptamer development for detection of the four most commonly used ACT partner drugs (Piperaquine, Mefloquine, Lumefantrine, and Amodiaquine). Our assay will filter out blood cells and provide a colorimetric readout of drug levels in recovered plasma via the aggregation of colloidal gold in the presence of target. In order to remove subjectivity associated with user interpretation of the assay, we will develop a smartphone application to quantify colorimetric readout, storing results along with location and patient information. We will design this device with constant input from clinicians and healthcare workers to ensure its feasibility for use in rural clinics in malaria-endemic settings.
Smith-Lemli-Opitz Syndrome (SLOS) is an autosomal recessive inborn error of cholesterol synthesis caused by mutation of the 7-dehydrocholesterol reductase (DHCR7) gene. This results in abnormal sterol levels; increased 7-dehydrocholesterol and decreased cholesterol. Although SLOS has a characteristic physical phenotype, with the most common finding being 2-3 toe syndactyly, there are also multiple behavioral abnormalities. These include cognitive deficits, anxiety, hyper-activity, sleep cycle disturbance, language impairment and autism spectrum behaviors. There are currently two main mouse models of SLOS; a homozygous null, Dhcr7Δ3-5/Δ3-5, and a model combining the null mutation and the common p.T93M missense mutation, Dhcr7Δ3-5/T93M. Unlike the null model, the hypomorphic Dhcr7Δ3-5/T93M mice can live to adulthood and are therefore suitable for behavioral studies. Two commonly used behavioral protocols, burrowing and nesting behavior, can be used to assess hippocampal function as it has been shown that hippocampal-lesioned mice engage in less burrowing and nest building than healthy controls. The burrowing test can additionally indicate anxiety as rodents exhibit defensive behavior by kicking the contents of the burrow to deflect intruders. The elevated plus maze and open field protocols are also further measures of anxiety and can additionally measure hyper-activity.

In a study of 3 to 5 month old Dhcr7Δ3-5/T93M mice it was observed that 3 month old female mutant mice spent significantly more time in the open zones of the elevated plus maze compared to gender and age-matched controls. These results also corresponded to the 3 month old female mice spending more time in the center of the open field compared to controls, suggesting decreased anxiety. This is unexpected as SLOS patients present with increased anxiety. A possible explanation for this apparent difference could be the result of another defect, such as decreased spatial awareness. The open field test also showed that the 3 month old female mutant mice covered more distance and moved at a higher speed than gender and age-matched controls, indicating hyper-activity in agreement with the behavior of SLOS patients. On average, Dhcr7Δ3-5/T93M mice burrowed and engaged in less nest building compared to age-matched controls. In all of the tests some variation was present but as only 75% of Dhcr7Δ3-5/T93M are found to have communicating hydrocephalus, other behavioral tasks, such as the Morris Water Maze, and histological analysis of the brain may be able to elucidate these results further.
Human papilloma virus (HPV) is the most common sexually transmitted infection in developing nations. HPV shows a diverse radiation of lineages (>10% genetic divergence) and sublineages (1-10% genetic divergence) that mirrors human migration patterns. Up to 50% of HPV cases may represent coinfections of multiple strains or sublineages. We demonstrate a method to detect and classify HPV isolates from a simulated coinfection sequenced on the Oxford Nanopore miniION using variation graphs and contemporary machine learning techniques. We describe a method for generating an HPV pangenome in the form of a variation graph and how such a structure can be used to produce reduced representations of alignments. These alignment vectors function as the inputs to a set of clustering and classification algorithms. Our model performs comparably to current best-practice approaches and is able to accurately classify reads into their strain of origin. Our approach is adaptable to a variety of clustering algorithms, takes only minutes to perform and in the future may be run in real-time during sequencing. Our results demonstrate the usefulness of variation graphs in a clearly translational context. Finally, we discuss the applicability of the method described to separate other complex mixtures of genomes.
Epigenetic remodeling of chromatin is fundamental to gene activation, particularly during development. Understanding the context of the epigenetic landscape, including the four-dimensional physical organization of functional genomic regions, is critical to developing targeted therapies and disease models. In developing motor neurons, the transcription factors Isl1 and Lhx3, in cooperation with their co-activator Ldb1, bind in a multi-protein complex to target genes, including the master regulator of motor neuron identity Mnx1. Our aim is to identify Isl1/Lhx3/Ldb1-dependent chromatin remodeling processes, both at the Mnx1 locus and genome-wide. Using a mouse embryonic stem cell system with inducible epitope-tagged factors, we start by epigenetic characterization of induced motor neurons with ChIP-Seq for histone modifications, coupled with ChIP-Seq and protein binding analysis of the Isl1/Lhx3/Ldb1 complex. To assess the changes in 3D genomic architecture during motor neuron development, we use chromatin interaction analysis (ChIA-PET) to determine long-range genomic interactions mediated through the Isl1/Lhx3/Ldb1 axis. Further, we introduce super-resolution microscopy data of both the complex and targeted genomic regions in individual cells to create nanometer-scale maps of complex architecture and chromatin organization. As our methodology reaches maturity, we will compare cell lines with mutant variants of Ldb1, as well as non-neuronal cell types (such as MEFs) that are not permissive of inducible remodeling, to infer general principles of epigenetic dynamics during early development. This multi-modal approach to interrogating 4D chromatin architecture during motor neuron differentiation has the potential to reveal mechanistic details of Isl1/Lhx3/Ldb1-induced transcriptional activation and chromatin remodeling in both specific and general genomic contexts, as well as establishing a conceptual and practical model for future de novo studies of functional genome organization.
### Abstract Title
Inhibition within starburst amacrine cell (SAC) network localizes SAC dendritic signaling and sharpens direction selectivity

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Direction selectivity (DS) in the mammalian retina originates in starburst amacrine cells (SACs), which send directionally-tuned inhibitory signals to DS ganglion cells (DSGCs). Previous work has shown that different regions of the SAC dendritic arbor operate independently and respond best to motion in the centrifugal direction (i.e., away from the SAC soma). SACs therefore constitute a powerful model for studying dendritic computations in a physiological context.

DS signaling in SAC dendrites appears to depend on both intrinsic dendritic properties and synaptic interactions within the DS network, but the relative impact of these mechanisms remains unclear. In addition, the elementary computational unit within SAC dendrites has not been identified. We have addressed these issues in mouse retina by imaging directional calcium signals from individual synaptic varicosities in SAC dendrites. We measured DS by comparing signals evoked by bars moving across the visual field in eight different directions. In addition, we mapped the visual receptive field properties of each varicosity to determine the spatial relationship between light-evoked inputs and outputs. In agreement with recent reports, we found that varicosities immediately adjacent to each other along the same dendritic branch exhibited similar directional preference. In addition, responses in adjacent dendrites arising from the same parent branch were highly correlated and exhibited similar DS. By contrast, responses in adjacent dendrites arising from different parent branches were poorly correlated and exhibited distinct directional preference.

Blocking SAC-SAC inhibition with GABAzine reduced the direction selectivity index (DSI) of responses in individual varicosities and increased correlations between adjacent branches, making DS tuning more similar on adjacent dendrites arising from different parent branches. GABAzine expanded the visual receptive field properties of individual varicosities, causing greater overlap between the receptive fields of adjacent branches. This effect was primarily due to blockade of SAC-SAC inhibition rather than feedback inhibition onto bipolar cell terminals, because GABAzine did not expand the receptive field properties of type 5 cone bipolar cells, which provide excitatory input to ON SACs.

Taken together, these results indicate that SAC-SAC inhibition compartmentalizes DS signaling within smaller regions of SAC dendrites, enabling finer discrimination of motion direction.
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<th>Abstract Title</th>
<th>Membrane protein folding via computer simulations</th>
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<td>Abstract Authors</td>
<td>Jan Domanski, Robert Best, Philip Stansfeld, Mark Sansom</td>
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Ab initio equilibrium sampling of membrane protein folding in lipid bilayers using all-atom simulations with explicit solvent and membrane is computationally challenging. The principal difficulty is the high viscosity of the bilayer, which is three orders of magnitude higher than that of water, as a result of which spontaneous peptide insertion or association is rarely observed on simulation-accessible time scales. Using replica exchange with solute tempering (REST) and all-atom MD simulations we find that lipid diffusion in the bilayer can be accelerated by 2-3 orders of magnitude. We use this approach to study a process underlying membrane protein folding, namely membrane insertion of a transmembrane helix. We show we can determine equilibrium insertion free energies for a series of model peptides containing charged residues, with the inserted fraction from simulation in good agreement with experiment. As a result, folding free energies at standard temperature can be recovered at much lower computational expense than a temperature replica exchange protocol with the same number of replicas. This result demonstrates the power of the REST method in studying membrane protein folding, and suggest that current protein and lipid force fields can accurately capture insertion equilibria of peptides in the membrane.
Artemisinin (ART)-resistant Plasmodium falciparum has emerged in western Cambodia and is spreading throughout Southeast Asia. Although mutations in kelch13 have been shown to confer resistance, resistance mechanism has not been defined. This project hypothesizes that autophagy, a homeostatic process to degrade waste and repair cells, is important for the parasite’s ability to survive exposure to ART and other antimalarial drugs. The purpose of this research project is to develop a novel FACS technique to measure autophagy in P. falciparum, as autophagy may be related to the ART resistance mechanism. At least 26 autophagic proteins exist in P. falciparum and recent publications suggest that autophagy occurs in the parasite in response to stressors. A genome wide association study comparing 782 isolates from Southeast Asia revealed that a nonsynonymous SNP in PF3D7_1012900− was significantly associated with slow parasite clearance rate in patients treated with an ART derivative (p=5.89E-7) and non-significantly associated with chloroquine resistance (p=0.0002). This gene encodes autophagy related protein 18 (Atg18), an important initiator of autophagy in mammalian systems. A recently developed assay to measure autophagy in mammalian cells involves the use of FACS to measure autophagosomes and lysosomes using pH-specific dyes. LysoID specifically stains lysosomes by staining low pH. Although lysosomes have not been specifically identified in P. falciparum, LysoID appears to stain the food vacuole—the acidic compartment of P. falciparum. CytoID stains autophagosomes in other organisms by staining intermediate pH and using Atg8/LC3 as an anchor. Previous studies observe an upregulation of autophagy in P. falciparum following a 6-hour amino acid starvation period. After a 6-hour incubation in amino acid free media, intraerythrocytic ring-stage parasites stained with LysoID had significantly higher mean fluorescence intensity than those that were incubated in complete media (979.3 vs. 555.5, p<0.001). In other systems, an increased LysoID signal alone can be used as a marker of autophagy induction. CytoID stained starved, intraerythrocytic parasites with slightly greater intensity than normal parasites, though this was not significant (63.0 vs. 58.1, p=0.27). This project demonstrates the potential for using FACS to measure autophagy in P. falciparum to determine if ART-resistant and ART-sensitive parasites have varying levels of autophagy.
Abstract Title: Chemically targeting the cancer cell at the DNA interface: Drugging the transcription factor FOXM1

Abstract Authors: Michael Gormally, Giovanni Marsico, Ganesha Rai, Christopher Lowe, Craig Thomas, David Maloney, Sam Michael, Dijana Matak-Vincovic, Ajit Jadhav, Anton Simeonov, and Shankar Balasubramanian

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Forkhead box M1 (FOXM1) is a transcription factor of considerable importance. Aberrant overabundance of FOXM1 through mutations in upstream regulators or gene amplification is now known to be a driving factor of most human cancers. Further, FOXM1 has prognostic value as expression correlates with severity of disease. Thus, chemical inhibition of FOXM1 has become a major goal. To address this need, we designed a novel in vitro assay to detect disruption of FOXM1 DNA binding. We executed a screen of 400,000 compounds from the NIH Molecular Library Small Molecule Repository, consisting of diverse drug-like molecules intended as starting points for medicinal chemistry lead development. After iterative and orthogonal counter screens, we ultimately identified the small molecule FDI-6 as a potent inhibitor of FOXM1. We characterized the perturbation in detail by biophysical analyses and confirmed that FDI-6 binds FOXM1 protein directly. The molecule was cytotoxic to multiple cancer cell lines (GI50 ≈ 10µM) and we demonstrated that the inhibitor displaces FOXM1 protein from promoters of target genes (AURKB, CCNB1, CDC25B) using an MCF-7 breast cancer model. To generalize the effect, we used chromatin immunoprecipitation and next generation sequencing (ChIPseq) to show that the inhibitor physically displaces FOXM1 from consensus binding motifs across the entire genome, reducing FOXM1 peaks by an average of over 60%. Transcriptome-wide expression profiling by RNAseq further confirmed that this displacement by FDI-6 selectively down-regulates the global FOXM1 transcriptional program, suppressing mitotic entry and cell-cycle progression. Importantly, we found that FDI-6 is specific for FOXM1 and has no effect on the expression of genes regulated by related forkhead family factors, which exhibit homology with the DNA binding domain of FOXM1. We are now evaluating the efficacy of this compound in allograft mouse models of FOXM1-driven breast cancer. Our study shows that the genomic interaction of this clinically important transcription factor can now be manipulated with small molecules to regulate the expression of key gene families. This improves our ability to probe transcription factor function, helps establish the oncogenic roles in different disease contexts and demonstrates clear potential for FOXM1 to be pursued as a clinical target in the future.
Magnetic resonance spectroscopic (MRS) imaging is a type of MRI which allows measurements of metabolites in vivo without harmful effects of ionizing radiation. However, it suffers from very low sensitivity and requires inadequately low resolutions or very long scan times. When MRS is coupled with dynamic nuclear polarization (DNP), a method by which molecules can be hyperpolarized to provide a greater than 10,000-fold increase in available signal, metabolic activity in living tissues can be probed in very short time courses. DNP has proved useful in measuring metabolic activity in tumors, but its potential in neuroinflammatory disorders such as multiple sclerosis (MS) has not yet been explored.

To investigate the potential of DNP to assess neuroinflammation, rats were inoculated against myelin oligodendrocyte glycoprotein, a protein found in white matter, and then surgically injected with cytokine or lipopolysaccharide in subcortical white matter to induce inflammatory lesions resembling those found in MS. Rats were injected with hyperpolarized pyruvate for serial imaging in which changes in pyruvate-lactate conversion were measured in the inflamed rat brain tissue. These were correlated with ultra-high resolution structural microscopic MR imaging and registered to histology. Preliminary results suggest a potential for DNP to be a useful method for assessing neuroinflammation in diseases such as multiple sclerosis, and as a potential benchmark for measuring drug efficacy in treating neuroinflammation.
Abstract Title | Characterising two novel protein interactors of Parkinson’s disease associated protein LRRK2
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Mutations in leucine-rich repeat kinase 2 (LRRK2) have been linked to familial and idiopathic Parkinson’s disease (PD). LRRK2 is a large enzymatic protein containing central core GTPase and kinase domains. Despite intensive study into the physiological function of LRRK2, a definitive consensus on how LRRK2 acts within cells and how pathogenic mutations compromise this function has yet to be achieved. Understanding protein-protein interactors of LRRK2 can offer key insight into downstream cellular mechanisms leading to neurodegeneration. In this study, we employed a yeast two-hybrid assay to characterise two novel protein interactors of the LRRK2 GTPase domain: ARMER and Arfaptin-2. In yeast, this interaction is conserved across disease associated mutants and protective variants of LRRK2. Quantitative yeast two-hybrid data has also highlighted differences in relative interaction strength across a range of mutants although these did not reach significance. This study is currently being expanded in order to investigate whether this interaction occurs in mammalian cells.
**Abstract Title**  
Protection against malaria at 1 year and immune correlates following PfSPZ vaccination

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An attenuated *Plasmodium falciparum* (Pf) sporozoite (SPZ) vaccine, PfSPZ Vaccine, is highly protective against controlled human malaria infection (CHMI) 3 weeks after immunization, but the durability of protection is unknown. We assessed how vaccine dose, regimen, and route affected protection over the course of 1 year in five vaccine groups. After 4 intravenous immunizations with 2.7x10⁵ PfSPZ, 6/11 (55%) vaccinated subjects remained without parasitemia following CHMI 21 week after immunization. Five non-parasitemic subjects from this dose group underwent repeat CHMI at 59 weeks and none developed parasitemia. Pf-specific antibody levels and Vγ9Vδ2+ T cells correlated with outcome following CHMI up to 21-25 weeks after immunization. However, antibodies waned substantially by 59 week when no vaccinated subject developed parasitemia. Pf-specific CD8 T cells were low in blood of PfSPZ-vaccinated subjects and similarly vaccinated non-human primates (NHP), but were ~100-fold higher in NHP livers. Our findings suggest that PfSPZ Vaccine conferred durable protection to malaria through long-lived tissue-resident T cells and that higher doses will further enhance protection.
The infectious organism *Mycobacterium tuberculosis* is capable of surviving in humans in a dormant, non-replicating state. These latent bacteria are thought to maintain their viability via metabolic adaptations, including a reductive tricarboxylic (TCA) cycle. In order to explore the importance of the reductive TCA cycle, we have developed a molecular probe to investigate the role of fumarase, a key enzyme in this pathway. We designed a fluorescence-based assay to detect fumarase activity, which we used to screen over 500,000 small molecules for enzyme inhibition. From this screen, we identified a compound that reproducibly inhibits the fumarase enzyme with an IC50 of 2 µM. A crystal structure of the compound bound to fumarase reveals a unique binding mode at a previously undocumented allosteric site. Although the human and the tuberculosis fumarase enzymes share a 53% sequence identity and conserved active site residues, the binding of this inhibitor to an allosteric site of the tuberculosis enzyme allows for complete selectivity over the human homolog. These discoveries provide a starting point for investigating the importance of the reductive TCA cycle in tuberculosis, and perhaps even provide an initial scaffold for drug development.
The accelerated advancement of genome-wide screening technologies such as RNAi and CRISPR has made it possible to generate extensive lists of genes implicated in the regulation of various signal transduction pathways. However, the further validation of the gene candidates generated by these studies is significantly hindered by the analytical challenge of correcting for the noise in the data that emerges from, both, the system being studied as well as the method used to achieve the gene perturbation. Additionally, given the varying sensitivities across different studies, the prevailing approach of using the highest scoring candidates as “hits” has led to widely divergent conclusions and limited general overlap across different screens studying similar pathways. These combined challenges have constrained the full potential of these technologies to identify broad and comprehensive networks of the regulatory factors associated with specific signaling pathways. To address this challenge we developed an analytical model that uses our recently developed CARD software platform to integrate genome-wide screening data with predicted protein-protein interaction data and canonical pathway databases while incorporating statistical corrections for possible noise in the experimental readout and varying readout sensitivity across different studies. For our study we used an siRNA system for genome-wide perturbation in human and mouse macrophages and the cellular response to bacterial LPS through the Toll-like Receptor (TLR) signaling system as our pathway. We demonstrate that our model selects candidate regulators that show a markedly increased validation rate in secondary screens than hits picked from simple score thresholds. Our analytical model provides a novel approach towards the interpretation of data from genome-wide studies and permits broader and more comprehensive insight into the network of genes and cellular process that regulate specific signal transduction pathways.
The placenta is a critical organ for the successful development of placental mammals. As such, improper function of the placenta through assorted insults can significantly affect fetal growth and lead to long-term systemic effects in the offspring. In placental malaria (PM), we potentially find two disparate mechanisms by which placental dysfunction may arise— inflammatory reactions elicited by sequestration of malarial parasites, and CSA-mediated adhesion of the parasites on the trophoblast via *Plasmodium falciparum* (Pf)EMP1 protein var2CSA, which theoretically hinders normal function and exchange.

As such, we examine the effect of *Plasmodium* infection on the placenta and the trophoblast in detail, using animal, cellular, and newly developed models to probe parasite-host interactions. First, we examine differential placental stresses experienced by C57BL/6 mice in first time infection and heterologous re-infection of *Plasmodium chabaudi* during pregnancy. This model is of interest for human Pf infection, as areas most burdened by pregnancy malaria are those where malaria is endemic. While most rodent models focus on first time infection, this unique model better captures human placental infections. Similar to what has been described in human populations, preterm birth was common only in first-time infections, while not in heterologous re-infections. Both groups showed varying signs of inflammatory and cellular stress; growth signaling was also significantly impacted in first-time infections, but less so in heterologous re-infections, while ER stresses seem to be more prevalent in re-infections. The data suggest that infections cause both groups to undergo inflammatory responses, albeit in slight different ways, while the effect on cellular stress and growth diverged more. These differential responses may result in the different fetal outcomes that are seen in this model.

However, animal studies of human PM are limited as rodent species of *Plasmodium* do not express var2CSA and have not conclusively been shown to adhere to the placenta, a key feature of Pf placental infection. To further investigate how var2CSA may directly impact trophoblast response to PM, and particularly how it directs the trophoblast to shape the host response to infection, the choriocarcinoma BeWo cell line is used; further, a novel murine-Pf model is developed by expressing a membrane protein of Plasmodium falciparum, a human parasite with little infectivity in rodents, on mouse erythrocytes. We seek to ask, for the first time, the extent to which a Pf driven PM pathology can be recapitulated in vivo solely by this interaction.
Glioblastoma is one of the most common and malignant forms of brain cancer. Despite knowledge of several of the major signaling pathways involved in the development and progression of the disease, the heterogeneous nature of the tumor makes it difficult to treat and contributes to an average survival of only 14 months after diagnosis even with surgical resection and chemo-radiotherapy. This disparity may be due to the clonal evolutionary dynamics of glioblastoma, demonstrated by the finding of multiple sub-clones within a single tumor fragment.

While the effects of chemotherapy, temozolomide in particular, on genomic mutation patterns have been studied, radiotherapy has not been studied as extensively. In order to better understand the effects of radiotherapy on the evolutionary genomics of glioblastoma, I am performing next-gen sequencing on spatially distinct, patient-derived tumor cell lines before and after treatment with radiotherapy in vitro. A small animal radiotherapy device will be employed to perform similar studies on orthotopic xenograft models generated from patient-derived cell lines. Future studies will also seek to determine if there is a difference in clonal evolution between tumor bulk cells and tumor margin cells which would suggest that different therapeutic targets are needed for treatment of these two distinct cell populations.

Due to the complexity of cancer genomics, particularly in tumors that display both intra- and inter-tumoral heterogeneity, computational modeling has the potential to serve an important role in individualized treatment planning. By utilizing the Bio Model Analyzer and F# modelling programs (Microsoft Research Ltd.), I am working to model the transcriptional signaling network and the spatial/physical pressures that contribute to glioma progression, respectively. Once an in silico model of glioblastoma is generated, then an individual patient’s genomic profile could potentially be incorporated into the model to predict their reaction to various combinatorial therapies.

By looking for key actionable mutations through genomic analysis of spatially distinct samples we can better understand the evolutionary genomics of glioblastoma. And by examining the effects of radiation on a tumor’s mutational pattern, including whether radiotherapy results in the emergence of resistant clones, we can provide necessary insight for the creation of more effective modeling tools and treatment regimens.
Within the mitochondrial reticulum of skeletal muscle, the I-Band segments (IBS) traverse the cell and form a contiguous matrix with the mitochondrial segments at the periphery (PS) of the cell. A tight electrical coupling via the matrix between the PS and IBS has been demonstrated. In addition, oxidative phosphorylation complexes that generate the proton motive force (PMF) are preferentially located in the PS, while Complex V, which utilizes the PMF, is primarily located along the IBS. This has led to the hypothesis that PS can support the production of ATP in the IBS by maintaining the potential energy available to produce ATP deep in the muscle cell via conduction of the PMF down the IBS. However, the mechanism of transmitting the PMF down the IBS is poorly understood. This theoretical study was undertaken to establish the physical limits governing IBS conduction as well as potential mechanisms for balancing the protons entering the matrix along the IBS with the ejection of protons in the PS. The IBS was modeled as a 300 nm diameter, water-filled tube, with an insulated circumferential wall. Two mechanisms were considered to drive ion transport along the IBS: the electrical potential and/or concentration gradients between the PS to the end of the IBS. The magnitude of the flux was estimated from the maximum ATP production rate for skeletal muscle. The major transport ions in consideration were H+, Na+ and K+ using diffusion coefficients from the literature. The simulations were run using COMSOL Multi-physics simulator. These simulations suggest conduction along the IBS via H+ alone is unlikely requiring un-physiological gradients, while Na+ or K+ could carry the current with minor gradients in concentration or electrical potential along the IBS. The majority of conduction down the IBS is likely dependent on these abundant ions; however, this presents a question as to how H+ is recycled from the matrix of the IBS to the PS for active extrusion. We propose that the abundant cation-proton antiporter in skeletal muscle mitochondria operates in opposite directions in the IBS and PS to permit local recycling of H+ at each site driven by cooperative gradients in H+ and Na+/K+ which favor H+ entry in the PS and H+ efflux in the IBS.
Parkinson’s disease is the second most common neurological disorder, affecting more than 1% of the world’s population aged 70 or older, and will only continue to grow in prevalence as the world’s population continues to age. Recently, studies into monogenic, inherited cases of Parkinson’s have led to a greater understanding of the etiology of the disease. It is now known that many of the genes associated with Parkinson’s directly impact autophagy and mitochondrial function. Mutant Parkin, VPS35, GBA and others cause autophagic phenotypes ranging from failed clearance of autophagosomes and ER stress to decreased mitophagy and impaired mitochondrial dynamics. However, how autophagic and mitochondrial dysfunction interplay is poorly understood. To this, these experiments sought to understand the effects of autophagic perturbation on mitochondrial function. BE(2)-M17 cells were treated with autophagy inhibitors and up-regulators for either 4 (acute) or 24 (chronic) h. After treatment, mitochondrial phenotypes such as respiration, ROS production, and turnover were examined. Preliminary data suggests that acute treatments have little impact on mitochondrial function while chronic treatment may decrease viability. In conclusion, further research is needed to fully elucidate the relationship between autophagy and mitochondrial function.
Abstract Title | Characterisation of the Injury Response in the Rodent Optic Nerve: Strategies to Improve Regeneration
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Retinal ganglion cells (RGCs) carry visual information from the retina to the brain via their axons. Like other central nervous system (CNS) neurons, RGC axons show only limited regeneration after injury or damage from disease. Successful regeneration requires an adequate stimulus to initiate axon regrowth, as well as a means of overcoming the growth-inhibitory environment of the adult CNS. A key aspect of this environment is the glial scar, which forms when astrocytes become reactive after injury and also includes activated microglia, macrophages, and extracellular matrix molecules such as chondroitin sulphate proteoglycans (CSPGs). The glial scar and its components inhibit growth of new axons and are thus targets for enhancing regeneration.

However, even following treatments that stimulate a significant population of RGC axons to regenerate beyond the injury site, pathfinding errors have been reported and functional reinnervation of central targets remains limited. Regenerating RGC axons must retrace a complex path that takes them out of the eye, along the optic nerve, through the optic chiasm, into the optic tract, and finally to visual targets in the brain. During development, specific guidance cues are expressed in a tightly regulated temporal and spatial manner to create a pro-migratory environment for newly projecting RGC axons. Changes in the expression of these cues in the adult optic pathway may account for the observed misguidance of regenerating RGC axons.

The aims of this PhD project are to characterise the cellular and molecular changes that occur throughout the optic pathway following acute injury. We hypothesise that CSPG upregulation accompanies reactive gliosis in the optic nerve, and that specific sulphation motifs on these proteoglycans mediate their inhibitory effect. Using arylsulfatase B, an enzyme that removes sulphate groups from the non-reducing end of CSPGs, we will alter the sulphation pattern of these proteins in an effort to reduce their inhibition of neuronal outgrowth. Additionally, we will perform proteomics analysis of protein expression changes at the optic chiasm, a critical navigation point for regenerating RGC axons, and seek to identify candidate proteins that mediate axon guidance and determine whether key developmental guidance cues are absent from the mature adult system. Combined, these efforts will enhance our understanding of the extracellular environment through which regenerating RGC axons must navigate after injury.
Mosquito hemocytes, the equivalent of vertebrate white blood cells, have an important role in the immune response to the malaria parasite, *Plasmodium*. However, much remains to be known with regards to both hemocytes basic biology and immunological effector mechanisms. Recently, hemocytes have been shown to mediate immunological memory-like responses in *A. gambiae* mosquitoes pre-exposed to malaria, despite being conventionally thought of as part of the innate immune system. This phenomenon is called immune priming, and was shown to be at least partially mediated by eicosanoids. In brief, we propose to utilize bulk RNAseq, single mosquitos bulk-RNAseq with single-cell protocols, and single-cell RNA-seq with index sorting to obtain biological insight on the way hemocytes modulate the *Anopheles* immune system, with particular attention on the mechanisms of immune memory. We will be uncovering the functional classes of the *Anopheles* mosquito hemocytes population and their development, with particular emphasis on their immunological memory (‘priming). High depth bulk RNAseq will provide us with a reference transcriptome of the *Anopheles gambiae* N’gousso strain, as well as overall responses of hemocytes, midguts and whole mosquitoes to *Plasmodium* infection or eicosanoid injection. Single-mosquito bulk RNAseq with scRNAseq protocols will allow us to disentangle inter-mosquito transcriptome variability from single cell data. Finally, single cell transcriptomics on the *Anopheles* hemocytes will allow us to uncover functionally relevant effector cell-population subtypes, molecular mechanisms of *Plasmodium* killing, and regulatory networks of immune memory. Longitudinal studies of the stimulated mosquito immune system by single hemocyte transcriptomics now offers an opportunity to overcome previous technical roadblocks and to describe hemocytes cell types and their developmental transition states for the first time, thus leading to a more comprehensive understanding of immunity in a medically important vector species. Furthermore, a similar immune priming mechanism has been recently described also in mammalian NK cells, and we hope our study will lead to further insight into innate human immunity mechanisms.
The TL1A-DR3 axis in immune complex-mediated nephritis

Systemic lupus erythematosus (SLE) is a complex autoimmune disease with multiple contributing genetic and environmental pathogenic factors and heterogeneous clinical manifestations, including organ-threatening kidney inflammation associated with immune complex (IC) deposition along the glomerular basement membrane (lupus nephritis). The kidney is populated by a rich network of heterogeneous tissue-resident mononuclear phagocytes that express Fc receptors and avidly internalize circulating and deposited ICs. TL1A (TNFSF15), a cytokine from the tumor necrosis factor (TNF) superfamily, is expressed at low levels in myeloid cells but is significantly induced following ligation of Toll-like receptors or Fc receptors by IgG ICs and co-stimulate T cells through its receptor DR3, another TNF receptor family member.

To determine whether DR3 could play a role in systemic autoimmunity, we studied the role of DR3 in the MRL/lpr mouse model of lupus which have a mutation in the TNF family receptor Fas (CD95,Tnfrsf6) that impairs its expression, causing a systemic autoimmune disease that mimics SLE observed in humans. DR3 deficiency significantly protected against the severity of glomerulonephritis while the other pathologies were unaffected. This was associated with a reduction in immune complex deposits in the glomeruli, T cell and myeloid cell infiltration in the kidney, and reduced levels of proteinuria. Although signaling through the TL1A-DR3 axis appears to be important for the onset of nephritis, the pattern of expression of these two proteins by the different actors of the disease has yet to be investigated.

Based on these findings we speculate that the accumulation of ICs in the kidney of lupus patients triggers the expression of TL1A by the resident cells, causing a local pro-inflammatory environment. Therefore, using mouse models of SLE and acute nephritis, we are comparing the different populations of mononuclear phagocytes residing in the kidneys of diseased versus healthy mice and their expression of TL1A to reveal which cells are contributing to the disease via secretion of this cytokine. Using cell-specific DR3 deficiency will allow us to measure the relative importance of this co-stimulation on the different cell subsets implicated in the inflammation.

Demonstrating that disrupting the TL1A-DR3 axis in lupus-prone mice reduces the inflammation in the kidneys while leaving the immune system fully capable of assuming its function would make this pathway a promising therapeutic target to prevent kidney failure in SLE patients.
The extrahepatic biliary tree is responsible for the drainage and storage of bile and digestive juices produced by the liver and pancreas. Diseases affecting this essential organ, such as primary sclerosing cholangitis and non-anastomotic biliary strictures, involve a failure to properly activate the regenerative response. Despite their importance, little work has focused on the mechanisms involved in regeneration of biliary tissues outside the liver. Even less is known about what differences, if any, may exist between different regions of the biliary tree.

The current research sought to identify progenitor cells from three regions of the human extrahepatic biliary tree: Gallbladder, Pancreatic Duct, and Common Bile Duct. Using a 3D organoid culture system, we have been able to derive progenitor cell lines from all three regions of the extrahepatic biliary system. These cells can be maintained in culture for over 20 passages and require Wnt signaling, cAMP activation, and TGF-β inhibition for long-term proliferation. Characterization of the cells suggests they express markers of adult stem cells (Lgr5, Prom1), biliary epithelium (EpCam, CK19, CK7, Sox9, HNF1β) and hepatocytes (HNF4α and TBX3) but lack mature cholangiocyte markers (SCTR, GGT).

Future work will explore the differentiation capacity of these cells and what differences may exist between the expanded cell populations from each anatomical region. Although results are still preliminary, these cells represent a promising model for studying biliary regeneration and have already led to an understanding of what signalling pathways may drive the proliferation of progenitor cells in the extrahepatic biliary tree.
### Abstract Title
Investigating the mode of action of novel inhibitors of HCV infection.

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Hepatitis C Virus (HCV) is a causative agent of liver cirrhosis, hepatocellular carcinoma and chronic hepatitis. The virus infects more than 170 million people worldwide and is responsible for some 280,000 deaths per year. The standard of treatment has been completely transformed since 2012 with the introduction of direct acting antiviral followed by combination therapies such as Harvoni and Viekira Pak.

A high-throughput screen was developed based on a robust cell culture system for HCV infection. Using the screen platform, novel hepatitis C inhibitors, with potentially novel targets, were identified. Categorization of these hits based on chemical structure features and modes of action revealed distinct chemotypes for chemical development and optimization. Amongst the hit compounds was chlorcyclizine HCl (CCZ), a currently approved over-the-counter anti histamine, that appeared to be a potent inhibitor of HCV replication. CCZ inhibits HCV infection in human hepatoma cell lines as well as primary human hepatocytes and inhibits the replication of HCV genotypes 1a and 2b in a chimeric mouse model. In chimeric mice engrafted with primary human hepatocytes CCZ significantly inhibits infection of HCV genotypes 1a and 2b without the emergence of drug resistance over 6 weeks. CCZ is a promising candidate for drug repurposing and development as a potentially cheap and effective treatment of HCV.

Preliminary investigations suggest that CCZ inhibits HCV replication by targeting the early stages of the viral life cycle, particularly viral entry into host cells, as opposed to most currently approved drugs which directly target viral replication machinery. Several lines of inquiry are currently simultaneously underway to identify the binding partners of CCZ and others, including photoaffinity labeling studies as well as other CCZ conjugated derivatives for use in chemical biology studies.
**Abstract Title**

Towards *In Vitro* Modelling of Intra-Embryonic Haematopoiesis

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During vertebrate embryogenesis, blood development occurs in three waves. The first two waves take place in the extra-embryonic yolk sac (YS) and give rise to transient blood; the third wave occurs independently in the intra-embryonic dorsal aorta (DA) and gives rise to haematopoietic stem cells (HSCs), providing the organism with lifelong blood. Numerous groups have attempted to produce HSCs from pluripotent stem cells (PSC). So far, none has succeeded. Importantly, in the DA, HSCs emerge from an arterial niche and require intricate signalling from the developing arterial cells; in contrast, YS haematopoiesis does not require an arterial identity.

Initially, we aimed to better characterise PSC-derived blood cells. Using lineage tracing studies and published protocols claiming to produce a YS-like wave followed by an independent DA-like wave of haematopoiesis from mouse ES cells, we demonstrated that cells in the first wave are actually progenitors of cells in the second wave, thereby refuting their independent origin. Using gene expression and flow cytometry analyses, we showed that blood progenitors obtained in the *in-vitro* second wave represent a haemogenic endothelium negative for markers of arterial identity, similar to YS haematopoiesis.

Our current aim is to develop serum-free culture conditions generating an arterial niche, thus more closely mimicking DA haematopoiesis to produce HSCs. To achieve this, we will differentiate an arterial endothelium that will then be further instructed towards a haematopoietic fate through timely delivery of appropriate growth factors. We have preliminary data that suggest levels of VEGFA in the culture medium of Flk-1+ endothelial progenitors regulate levels of arterial commitment genes at the expense of haemogenic endothelial genes, thus pointing to VEGFA levels at this stage as critical in the differentiation of an arterial endothelial niche before the induction of haematopoiesis.

In conclusion, the two haematopoietic waves produced by published differentiation protocols represent YS haematopoiesis. Our approach to producing HSCs *in vitro* is to recapitulate the intra-embryonic wave of haematopoiesis.
Malaria remains a devastating disease, responsible for nearly 500,000 deaths in 2015. The disease is caused by obligate intracellular parasites of the genus *Plasmodium*, with *Plasmodium falciparum* being the most virulent and deadly of the five species that infect humans. Clinical manifestations derive from repeated cycles of invasion of, asexual replication within, and exit from erythrocytes. Disrupting this asexual intraerythrocytic cycle would prevent development of clinical disease.

Rhoptries are specialized apically-located protein-containing organelles seen in invasive daughter merozoite forms of the parasite. Whilst the function of most rhoptry proteins remains unknown, rhoptry release temporally coincides with erythrocyte invasion. RhopH3 is one component of the high molecular weight (HWM) rhoptry complex, together with RhopH1/clag and RhopH2. This ~110 kDa protein has been hypothesized to play a role in erythrocyte invasion. Failed attempts at conventional genetic knockout of *PfRhopH3* suggested this gene to be essential for *P. falciparum* survival.

We have used a combination of the Cas9 and DiCre systems to achieve conditional disruption of *PfRhopH3*. This confirmed the essential nature of the gene and resulted in two distinct phenotypes. As hypothesized, the ability of mutant parasites to invade erythrocytes was significantly reduced. Those mutant parasites still capable of invading were unable to progress through the intraerythrocyte cycle to form multinuclear schizont-stage parasites, instead stalling as mononuclear trophozoite-stage parasites. These stalled parasites appear competent as regards protein export. However, we have shown that stalling occurs due to deficient nutrient import. This indicates a dual role for RhopH3 in asexual blood stage parasites: in invasion and in nutrient import.
Intravenous immunization with highly purified, radiation-attenuated *Plasmodium falciparum* (Pf) sporozoite (SPZ) vaccine (PfSPZ Vaccine) is safe, immunogenic and confers high-level protection against controlled human malaria infection (CHMI). Protection was associated with a dose-dependent increase in PfSPZ-specific antibodies and CD4+ T cell responses. Heretofore, multi-parameter flow cytometry has been used to characterize the phenotype, magnitude and quality of PfSPZ-specific T cell responses following vaccination or infection. To substantially expand the analysis of such responses, we performed high-resolution, quantitative transcriptome analysis of PfSPZ-specific CD4+ T cells using Fluidigm 96.96 Dynamic Arrays. Accordingly, PfSPZ-specific CD4+ T cells expressing the costimulatory marker CD154 (CD40L) were sorted following *in vitro* activation with sporozoites.

We first analyzed the gene signature of PfSPZ-specific CD4+ T cells from vaccinated subjects two weeks after their final immunization and prior to CHMI vs. nonvaccinated control subjects two weeks after primary infection. There was evidence of considerable T cell heterogeneity following vaccination, as we detected gene expression of cytokines and transcription factors associated with a spectrum of T-helper (Th) subsets, such as Th2, Th17 and TFH. Blood transcription module analysis revealed a distinct transcriptional signature of natural infection composed of genes associated with T cell activation, differentiation and migration. High expression of TBET, ICOS, and CXCR3 were among the largest drivers of a primary infection signature, likely due to an increased antigen load present in the liver following CHMI vs. PfSPZ immunization.

In order to understand factors that may play a role in protection, we examined differentially expressed genes between protected and unprotected vaccinees. While only 10 protected and 2 unprotected vaccinated subjects were available for this analysis, thirteen genes were differentially expressed between the two groups. Of note, *IL21* expression was increased in protected vs. unprotected subjects prior to challenge. The subject-level mean of *IL21* gene expression significantly correlated (Spearman rank correlation: ρ = 0.69, p = 0.004) with antibodies titers against the circumsporozoite protein (CSP), the major surface protein on PfSPZ. We tested the hypothesis that malaria-specific CD4+ TFH cells play a role in PfSPZ-mediated protection in a second, independent clinical study. Utilizing the same single-cell data acquisition and processing techniques, we found a similar enrichment of *IL21* gene expression in protected vs. unprotected subjects, which correlated with anti-PfCSP antibody titers. *IL21*-expressing cells predominantly coexpressed *BCL6* and *TBET*, alone or in combination.

Overall, this analysis should advance our understanding of the striking heterogeneity of CD4 T cell responses by a parasite vaccine and highlight how TFH CD4+ T cells may influence protection against human malaria infection.
**Purpose:** The inability of the retina to detect/transmit light-triggered signals due to dysfunction or death of photoreceptor cells is manifested in incurable blinding conditions. The purpose of this research is to identify novel cone-enriched factors conserved in zebrafish, mouse and human macula and characterise their involvement in cone development, function and survival.

**Methods:** Microarray analysis of cone photoreceptors of TG(3.2TαCP:EGFP) zebrafish and RNAseq data of photoreceptors of the cone dominant Nrl-/- mouse and rod dominant Nrl-GFP mouse were compared to identify conserved cone-enriched factors. Human retina and macular RNAseq data was analysed to confirm evolutionary conservation. Genes were ranked on cone enrichment. PCR was performed in wild type (Tü) zebrafish eyes at developmental stages: 3, 4 & 5 days post-fertilization (dpf). Fluorescent in situ hybridisation (FISH) of high-ranking factors was performed on developing and adult TG(3.2TαCP:EGFP) zebrafish. Gene knockdown of clul1, one of these high-ranking genes was performed using morpholino technology. Morphants visual behaviour was assessed using the optokinetic response, and retinal integrity examined using light microscopy. We are currently developing CRISPR-Cas9 mediated gene knockout for our 5 highest-ranking genes.

**Results:** Upon ranking based on enrichment in zebrafish and mice, twentyseven novel, conserved, cone-enriched genes were selected for further analysis. These factors were conserved and enriched in human retina. High-ranking genes were confirmed to be present during development at 3, 4 and 5 dpf in wildtype (Tü) zebrafish, with the genes clul1 and es1 demonstrating an incremental increase in expression. FISH revealed the gene clul1 was specifically expressed in adult zebrafish cone photoreceptors. Knockdown of the gene clul1 resulted in a significant impairment of visual behaviour without substantial morphological differences in the retina.

**Conclusions:** Twentyseven novel, conserved cone photoreceptor-enriched factors were identified and spatiotemporal expression elucidated. Knockdown of clul1 indicates it is not required for normal cone photoreceptor morphogenesis but is required for cone photoreceptor mediated visual function. Cone photoreceptor specific expression is aiding the development of CRISPR knockout models to elucidate the role these factors play in cone morphogenesis and function.
From learning maths to playing golf, skills are necessary for success across the lifespan. Understanding the mechanisms that subserve skill learning and developing methods to expedite the learning process can therefore have a huge impact on education to rehabilitation. Feedback (FB) given during skill learning improves immediate performance and aids long-term memory formation. However, neither the specific effects of reward (REW) and punishment (PUN) nor generalizability of those effects across different types of skill is well understood. The present study investigated the influence of REW and PUN on learning two different tasks: a serial reaction time task (SRT), requiring participants (HVs) to press a sequence of buttons according to visual cues, and a force-tracking task (FTT), requiring HVs to squeeze a bar to move a cursor on the screen to track a moving target. To monitor brain activity, 72 HVs had MRI before, after, and during training. HVs received REW, PUN or control (CONT) FB. In SRT, accuracy and reaction time determined FB, which was given after each button press. In FTT, FB was given when the HV was within a certain error margin. In REW, HVs were told if their performance was improving. In PUN, HVs were told when their performance was getting worse. In CONT, HVs received FB unrelated to their performance. We examined performance during learning and tested retention at 1 hour, 24 hours, and 30 days after the learning finished. The effect of FB on performance during learning differed between the tasks. For SRT, HVs in PUN condition responded faster than those in REW or CONT with minimal impairment in accuracy. In the FTT, REW had the least error, equating with better performance. In contrast to performance, there was no difference between the FB conditions on retention for either task. Interestingly, while the tasks differ dramatically, they elicit nearly identical patterns of brain activity after training. Areas of the brain that deduce statistical regularities increase communication with areas that drive motor output and integrate visual information, suggesting that the learning relies on binding of visual and motor information. Retention of skill memory correlated with the strength of this communication after training. These results suggest that FB influences performance rather than long-term memory and relies on the integration of multiple types of information after training.
**Abstract Title**  
The genetic architecture of metabolic response in skeletal muscle expression

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Type 2 diabetes (T2D) is a complex disease that results from a combination of genetic and environmental factors, including physiological responses to lifestyle choices. T2D is characterized by dysfunction in insulin secretion and insulin resistance. The genetics of T2D is well studied, with >80 known loci from genome wide association studies. However it is unclear how the combination of genetics and physiological factors influence the etiology of T2D.

As part of the Finland United States Investigation of NIDDM Genetics (FUSION) Study, we analyzed 278 individuals with metabolic measures (glucose, insulin, and other blood metabolites), genotypes, and muscle biopsies (mRNA-seq). A considerable challenge in RNA-seq studies is the presence of technical (e.g. batch effects) and biological (e.g. effects of physiological state) variation. Furthermore, because technical and biological variation can have widespread effects across many genes, methods that account for these confounders struggle to distinguish technical from biological signals. We developed a novel technique that iteratively fits random effect covariance from gene expression data, while conditioning on biologically meaningful covariates.

We used this method to remove confounders but preserve metabolic signals in gene expression, and find it greatly improves power to identify genes sensitive to metabolic measurements – most notably insulin. We then characterized genetic effects of two types – genetic control of gene expression (eQTL), and a genotype dependent relationship between gene expression and metabolic measurements. In heterozygote individuals, we also explored the impact of these genetic responses using allele specific expression. Together, these methods reveal new regulatory loci and mechanistic insights into the response of skeletal muscle to T2D progression.
Interest in the regulation, transport, and localization of coding and noncoding RNA has steadily increased over the past few decades, and practical methods for imaging cellular RNAs are necessary to elucidate the various functionalities of this class of biomolecules. While several methods have evolved to visualize RNAs, each technique possesses certain limitations that make it non-ideal for live cell imaging. Recently, a new class of RNA fluorescent tags analogous to green fluorescent protein (GFP) was developed. These RNA mimics of GFP are comprised of an RNA aptamer which induces the fluorescence of a bound exogenous small molecule chromophore. Spinach, the first fluorogenic RNA aptamer of this class characterized crystallographically, demonstrates much promise for in vivo imaging both RNA and cellular metabolites, but exhibits spectral and photophysical limitations under cellular imaging conditions. Crystallographic analyses of Spinach revealed a G-quadruplex structural core which is responsible for binding and inducing the fluorescence of 3,5-difluoro-4-hydroxybenzylidene (DFHBI). Preliminary studies suggest the aptamer’s fluorescent properties are tightly coupled to the electronic environment surrounding the chromophore and G-quadruplex, suggesting a promising avenue to spectrally tune the fluorogenic RNA aptamer. We will use artificial and naturally occurring post-transcriptionally modified nucleotides to pursue this line of research and probe the fluorescent properties of Baby Spinach. This will provide valuable insight into developing multipurpose fluorogenic RNA aptamers with expanded fluorescent properties. Furthermore, we will develop a system to selectively introduce post-transcriptional modifications in vivo, generating a genetically encoded approach to spectrally tune the fluorogenic RNA aptamer.
It is well known that regional changes in cardiac function precede global changes such as ejection fraction and myocardial mass, and therefore quantification of regional function has become a central goal of cardiology. Despite extensive studies demonstrating their usefulness, these techniques have unfortunately not migrated from the research setting to clinical practice due to lengthy acquisition and analysis procedures. Recent advances in cardiac computed tomography (CCT) have allowed volumetric time-series covering the entire cardiac cycle to be acquired in a single heart beat at clinically acceptable (<2mSv) radiation doses and high temporal resolution (50ms). Here, we propose an algorithm for quantifying functional information from contrast-enhanced CCT and share initial experience in a canine infarct model.

The bloodpool of the left ventricle and atrium is identified by thresholding the image, “opening” by mathematical morphology to separate from the bone, and selecting the largest connected component. The boundary of the bloodpool, corresponding to the endocardium, is then found using the marching cubes algorithm. A coarse triangular mesh was constructed which defined a Loop subdivision surface modeling the structure of the endocardium. The mesh was initialized to roughly coincide with the boundary candidates, and the mesh model was registered to the data using the Levenberg-Marquardt algorithm. The parameters of the optimization were the positions of the vertices of the boundary candidates, and the residuals were the distances from a sampling of points on the subdivision surface and the nearest boundary candidate. The optimization was performed globally over all cardiac phases, avoiding the accumulation of error which can occur in “track-to-last” algorithms, where each phase is initialized by the solution of the previous frame. The global approach additionally allows for the program to be parallelized. The distance between corresponding mesh vertices in adjacent frames was added as a regularization penalty, in order to enforce anatomical correspondence between frames. The software was implemented by the author in c++ using ITK for image processing, VTK for visualization, Qt for the graphical user interface.

The algorithm was tested in 14 canine subjects who had undergone surgical left anterior descending (LAD) occlusion lasting 90 minutes. Circumferential, longitudinal, and area strain (all measures of regional cardiac function) were calculated according to the American Heart Association seventeen-segment model. Validation against MRI gold standard is forthcoming.
Natural Killer (NK) cells are cytotoxic lymphocytes of the innate immune system that are crucial for defense against viruses and cancer. It is becoming increasingly more apparent that immune cell function and metabolic status are integrally linked. Cytokine activated NK cells undergo mTORC1 (mammalian target of rapamycin complex 1) mediated metabolic reprogramming, which is crucially linked to their ability to undergo blastogenesis and produce effector molecules. Glycolysis can function as a reservoir of biosynthetic precursors for the synthesis of nucleotides, lipids and amino acids, thus promoting anabolic processes. Pyruvate Kinase is the last enzyme of glycolysis and is required for the synthesis of pyruvate and ATP from phosphoenolpyruvate (PEP) and ADP. Natural Killer cells express two isoforms of this enzyme, Pyruvate Kinase Muscle 1 (PKM1) and Pyruvate Kinase Muscle 2 (PKM2). PKM1 is a highly processive tetrameric isoform with a low Km for PEP. In contrast, PKM2 can be found as an active tetramer or and inactive dimer with a high Km for PEP. This inactive dimer can act to limit glycolytic flux resulting in increased levels of glycolytic intermediates that can be used for biosynthesis and cell growth. Therefore, dimeric PKM2 can be viewed to promote anabolic cellular processes. Dimeric PKM2 is also reputed to have nuclear functions and has been demonstrated to interact with key transcriptional regulators such as HIF1α in macrophages. The data shows that in NK cells mTORC1 signalling promotes the phosphorylation of PKM2 on Tyr 105; phosphorylation of this site is associated with monomeric/dimeric PKM2. Both inhibition of mTORC1 and pharmacologically inducing tetramerisation/activation of PKM2 inhibits NK cell growth and prevents normal blastogenesis. Activation of PKM2 results in an immediate increase in glycolytic flux. However, prolonged activation of PKM2 decreases the expression of the glycolytic transcription factor c-myc resulting in the down-regulation of the glycolytic machinery of the cell, that is glucose transporters and glycolytic enzymes. This preliminary data argues that dimeric PKM2 functions as a key switch towards anabolic metabolism in cytokine stimulated NK cells.
Exaggerated beta range (15-30 Hz) activity is observed in the motor circuits of Parkinson’s disease (PD) patients and animal models and this activity has been hypothesized to contribute to motor dysfunction in PD. Further, it has been shown that deep brain stimulation (DBS) and L-DOPA help to relieve these symptoms by disrupting this pathological beta frequency activity. DBS and L-DOPA have also been shown to alleviate Parkinsonian pain. Nonetheless, the existence (and potential consequences) of pathological beta in cognitive and pain circuits is not yet clear. A recent study from our lab failed to show exaggerated beta rhythms in the medial prefrontal cortex of PD rats, but did observe such activity in the motor cortex and subthalamic nucleus (STN) during a treadmill walking task. Anatomical studies in rats have shown that the ventral medial thalamus (VM) receives basal ganglia output and projects to the motor cortex as well as the anterior cingulate cortex (ACC). Inhibition of the VM has also been shown to disrupt excessive beta rhythm in the basal ganglia-thalamocortical circuit. The ACC is associated with a broad range of functions including cognition and pain perception. In view of these functional and anatomical evidences, we hypothesized that the ACC also exhibits excessive beta rhythms in PD and that the basal ganglia-thalamic-ACC circuit may thus be involved in PD symptoms as diverse as pain and cognitive impairments.

This study used the 6-OHDA lesioned, hemiparkinsonian rats performing a treadmill walking task to compare synchronized STN local field potential (LFP) activity with VM and ACC activities. Electrode bundles were implanted in the STN, VM, and ACC of rats with unilateral dopamine cell lesions. LFP and spiking activity were recorded during epochs of treadmill walking and rest on a circular treadmill. As previously shown, in dopamine-cell depleted rats, STN and VM exhibited an increase in the high beta range 30-36 Hz LFP power spectra during treadmill walking compared to control animals. Interestingly, these animals also showed a similar increase in ACC high beta, 30-36 Hz LFP power during treadmill walking. LFP in this structure exhibited robust increases in coherence with LFP activity in the same frequency range in the STN and VM, relative to non-lesioned control rats. Administration of therapeutic L-DOPA was shown to reduce beta power and coherence in the STN, VM, and ACC while restoring walking ability. These results suggest that the ACC integrates activity from the STN and VM in a manner that varies with frequency, behavioral state, and the integrity of the dopamine system in the behaving parkinsonian rat. This may help us gain further insight into the significance of pathological beta rhythms in PD and its ramifications in motor, cognitive, and pain circuits.

References:
Reduced levels of full-length survival motor neuron (SMN) protein lead to the degeneration of motor neurons in spinal muscular atrophy (SMA). SMN is likely involved in small nuclear ribonucleoprotein assembly, axonal mRNA transport, and neuromuscular junction maturation. SMA is caused by deletions and other mutations in the SMN1 gene, with retention of one or more copies of the homologous gene, SMN2. Increasing the number of SMN2 transcripts has the potential to increase full-length SMN protein levels. Current therapeutic strategies include splice modulation therapy of SMN2 pre-mRNA to produce more full-length transcripts, and the use of small molecules to increase SMN2 promoter activity. To date, the mRNA degradation pathway has not been well explored as an avenue for increasing SMN levels. The 3’UTR of SMN transcripts contains AU-rich elements, which are considered mRNA destabilizing features, although recent work suggests that in certain cases AU-rich elements increase transcript stability. We hypothesize that the binding of antisense oligonucleotides (ASOs) to AU-rich elements will block the degradation machinery’s access to these sites, thereby increasing the total pool of SMN2 transcripts and facilitating an increase in SMN protein levels. To test this hypothesis, we designed three 20-mer ASOs to target different AUUU(U)A sequences in the 3’UTR of SMN2 transcripts. To determine the effect of the ASOs on transcript levels, we are using a luciferase reporter assay system in HEK293T cells, allowing expression of the Renilla luciferase gene under the regulation of the SMN2 3’UTR. When normalized to Renilla expression in cells transfected with a scrambled oligonucleotide sequence, an increase in luciferase activity is interpreted as ASO-mediated stabilization of SMN2 RNA, while a decrease in luciferase activity is interpreted as ASO-mediated degradation. We find that all three ASOs modestly increase luciferase signal, suggesting that the transcript AU-rich elements indeed influence protein steady-state levels. Preliminary data suggests treating with a combination of ASOs has an additive effect. In addition, using RT-PCR and Western blotting, we will assess the effect of the ASOs on endogenous SMN levels in SMN-deficient patient cells. The results of these experiments should indicate whether AU-rich elements are important determinants of the stability of the SMN2 transcript and a viable target for increasing SMN levels.
Transcriptional enhancers are frequently bound by a set of transcription factors that collaborate to activate lineage-specific gene expression. Recently, it was appreciated that a subset of enhancers comprise extended clusters dubbed stretch- or super-enhancers (SEs). These SEs are located near key cell identity genes, and enriched for non-coding genetic variations associated with disease. Previously, SEs have been defined as having the highest density of Med1, Brd4 or H3K27ac by ChIP-seq. The histone acetyltransferase P300 has been used as a marker of enhancers, but little is known about its binding to SEs. We establish that P300 marks a similar SE repertoire in embryonic stem cells as previously reported using Med1 and H3K27ac. We also exemplify a role for SEs in mouse T helper cell fate decision. Similarly, upon activation of macrophages by bacterial endotoxin, we found that many SE-associated genes encode inflammatory proteins that are strongly up-regulated. These SEs arise from small, low-density enhancers in unstimulated macrophages. We also identified expression quantitative trait loci (eQTL) in human monocytes that lie within such SEs. In macrophages and Th17 cells, inflammatory SEs can be perturbed either genetically or pharmacologically thus revealing new avenues to target inflammation. Our findings support the notion that P300-marked SEs can help identify key nodes of transcriptional control during cell fate decisions. The SE landscape changes drastically during cell differentiation and cell activation. As these processes are crucial in immune responses, SEs may be useful in revealing novel targets for treating inflammatory diseases.
Osteosarcoma is the most common primary bone malignancy with peak incidence during adolescence. As a diagnostic category, osteosarcoma is both genetically complex and heterogeneous. When combined with the low incidence of sporadic osteosarcoma in the general population, dissecting the pathways and mechanisms underlying the development of osteosarcoma remains challenging.

Rothmund-Thomson syndrome is an inherited cancer predisposition syndrome caused by germline mutations in the RECQL4 helicase. More importantly, roughly 30% of RTS patients develop osteosarcoma. We seek to gain insight into the malignancy through studying the role of RECQL4 in tumorigenesis.

RECQL4 is one of five members of the RecQ helicase family in humans. Comprising an N-terminal SLD2-homology domain and a C-terminal helicase domain, RECQL4 is believed to have multiple functions in DNA replication and repair. It has been shown that the unique N-terminal SLD2-homology domain is required for the initiation of DNA replication and consequently cell viability. However, the functions of the C-terminal helicase domain remain unclear. Recent studies have reported a preference for helicase domain binding of branched DNA substrates, association of RECQL4 with components of homologous recombination repair such as RAD51, and sensitivity of human cells expressing C-terminal truncation mutants to ionizing radiation.

Based on these lines of evidence, we propose that RECQL4 is associated with the DNA replication machinery beyond the initiation phase where it functions as a sensor for DNA damage such as double strand breaks. Upon replication fork encountering such lesions, RECQL4 then engages and unwinds newly synthesized DNA to generate the ssDNA substrate that ultimately recruits the homologous recombination machinery that facilitates DNA repair and fork restart.

Currently ongoing work follows two parallel tracks: 1) we wish to study the location of RECQL4 throughout the cell cycle, in particular during S-phase. Work will be done to determine the interaction partners of RECQL4 both during normal DNA replication as well as upon challenge with DNA damaging agents. 2) We will explore the effects of RTS RECQL4 mutations on the functions of the C-terminal helicase domain. Work done here will determine whether cells expressing these mutants show defects in DNA repair mechanisms, specifically homologous recombination repair associated with stalled DNA replication.

Finally, we hope to reconnect work on RECQL4 and genome stability with previous work done at the NIH. In particular, we wish to explore whether defects associated with pathways involving RECQL4 contributes to the high incidence of structural alterations in sporadic osteosarcoma such as TP53 intron 1 translocations.

By studying Rothmund-Thomson syndrome and the functions of RECQL4 in genome maintenance, we hope to shed light on mechanisms which may be shared in the pathogenesis of both RTS-associated and sporadic osteosarcoma ultimately leading to a better understanding of the development of this malignancy.