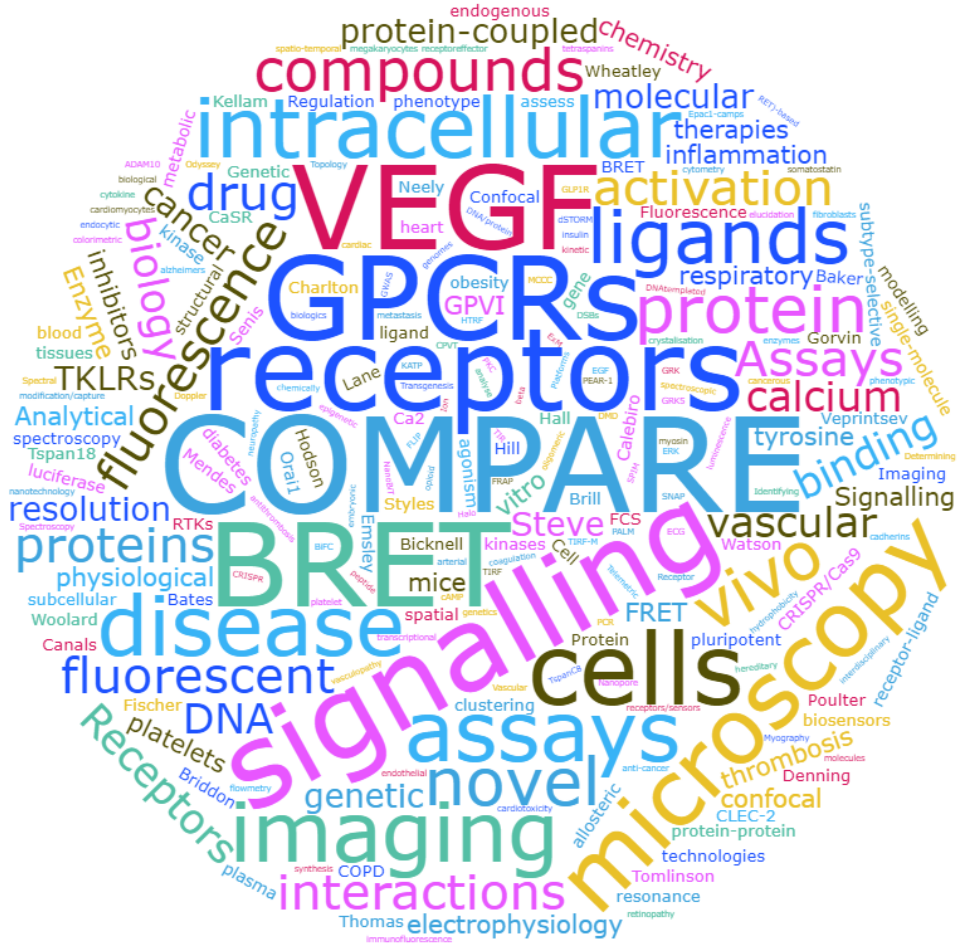
COMPARE
CENTRE OF MEMBRANE PROTEINS AND RECEPTORS



PI TECHNOLOGY SPOTLIGHT 2019

COMPARE PI: Jillian Baker

Technologies used

- analytical molecular pharmacological techniques for detailed studies of ligand affinity and ligand efficacy, including:
  - Whole cell ligand binding
  - cAMP
  - calcium
  - inositol triphosphate
  - MAP Kinase
  - CRE-reporters
  - SRE-reporters

COMPARE PI: Jillian Baker

Main areas of research

- Understand the detailed molecular pharmacology of G-protein coupled receptors in order to:
  - understand why certain medicines have the side effects they do
  - develop approaches to make medicines devoid of these side effects
  - develop approaches for novel medicines for conditions for which there are currently no or few therapeutic options

Biological questions to be addressed

- How and why do some ligands bind to a certain receptors but not a closely related receptor? What important receptor-ligand interactions need to occur to determine receptor selectivity?
- Why is it that some ligands are able to stimulate responses whilst others are not? What important receptor-ligand interactions are required for efficacy?
- How can we change current medicines/molecules or create new ones without the undesired interactions?

IN PARTNERSHIP:
The Universities of Birmingham and Nottingham

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COMPARE PI: David Bates

Technologies used

- In vivo technologies
  - Measurement of vascular permeability
    - laser scanning confocal microscopy
    - Epifluorescence
    - Fundus fluorescence angiography
    - optical coherence tomography
    - light microscopy
  - Laser Speckle imaging of blood flow.
- Nano-BRET-based approaches to monitor kinase-substrate interactions.
- Cell biology assays and immunofluorescence imaging.
- Assays to investigate receptor-mediated intracellular signalling.

COMPARE PI: David Bates

Main areas of research

- VEGF and VEGF Receptors
- Splicing factor kinase function in health and disease
- Vascular growth and revascularisation in ischemia, cancer and diabetes (retinopathy, nephropathy, vasculopathy and neuropathy
- Cancer progression and growth

Biological questions to be addressed

- How does splicing factor kinase change cell function?
- How is VEGF splicing regulated, and how does this drive phenotypic changes resulting in disease?
- How do cell signalling pathways in cancer cells drive cancer growth and metastasis?

COMPARE PI: Roy Bicknell

Technologies used

- Genomic analysis of RNA: microchips and RNAseq
- Generation and characterisation of monoclonal Abs
- Genetic modification of T cells
- Assays to investigate receptor-mediated intracellular signalling
- Analysis of endothelial cell gene expression under different shear stress
- Confocal and light microscopy
- Animal models of disease: cancer, atherosclerosis and arthritis
- Generation of genetically modified mice

COMPARE PI: Roy Bicknell

Main areas of research

- Biology of type 14 C-type lectins
- Identification of tumour targets lying within the vasculature
- Role of CLEC14A in cancer, atherosclerosis and arthritis
- Development of CAR modified T-cells to tumour vascular targets

Biological questions to be addressed

- How is CLEC14A expression regulated by shear stress
- How do the type 14 C-type lectins signal into the cell
- Can targeting CLEC14A and its ligand multimerin2 develop novel therapies for cancer, atherosclerosis and arthritis

COMPARE PI: Steve Briddon

Technologies used

- Fluorescence fluctuation spectroscopy approaches (fluorescence correlation spectroscopy (FCS) and related techniques) to quantify the speed of movement, number and brightness of single fluorescent particles (e.g. receptor complexes, ligand-receptor complexes). Live cell and solution based.
- Confocal microscopy (including F-techniques: FRAP, FRET, FLIP etc.) and other advanced microscopies to image distribution and dynamics of receptor and receptor-ligand complexes, and functional responses (e.g. calcium)
- (TIRF/super-resolution)
- Analytical pharmacological assays for receptor-ligand interaction and cellular signalling (including BRET, reporter gene and calcium)
- Fluorescent ligand development

COMPARE PI: Steve Briddon

Main areas of research

- Advanced imaging approaches to investigate receptor organisation, trafficking and stoichiometry and its effect on pharmacology.
- Molecular pharmacology of G protein-coupled receptors.
- The effect of receptor heterocomplexes, signalosomes and scaffolding proteins on subcellular pharmacology.

Biological questions to be addressed

- How are receptors organised in the cell membrane and how does their location and associated proteins affect their pharmacology?
- Does this organisation (and therefore pharmacology) differ between cell types expressing these proteins at endogenous levels?

COMPARE PI: Alex Brill

Technologies used

- Flow disruption-induced models of deep vein thrombosis in mice
- Murine models of thrombosis in different vascular beds
- Intravital microscopy
- Blood cell isolation, activation and co-culture
- Various types of cell and tissue staining and microscopy
- Flow cytometry

COMPARE PI: Alex Brill

Main areas of research

- Mechanisms of deep vein thrombosis initiation
- Interplay between thrombosis and inflammation
- Identification of novel target to prevent venous thrombosis

Biological questions to be addressed

- Role of mast cells in deep vein thrombosis
- Role of neutrophil extracellular traps and their interplay with other inflammatory processes in the context of venous thrombosis

COMPARE PI: Davide Calebiro

Technologies used

- Development and use of biosensors based on fluorescence resonance energy transfer (FRET) to monitor GPCR signalling in living cells and tissues
- Generation of genetically modified organisms (mice, flies) expressing fluorescent receptors/sensors (e.g. Epac1-camps mice, CRISPR/Cas9)
- Single-molecule microscopy and single-particle tracking in living cells (e.g. to investigate diffusion and protein-protein interactions), *d*STORM, PALM
- Development of advanced computational algorithms to analyse single-molecule data (protein-protein interactions, diffusion, etc.)
- Assays to investigate receptor-mediated intracellular signalling

COMPARE PI: Davide Calebiro

Main areas of research

- Molecular pharmacology of G protein-coupled receptors
- GPCR trafficking and signalling at intracellular sites
- Innovative microscopy methods to investigate the spatiotemporal organization of GPCR signalling in living cells
- Alterations of GPCR signalling in endocrine/metabolic (and cardiovascular) diseases

Biological questions to be addressed

- Where do GPCRs signal in our cells? (nanodomains on the plasma membrane, intracellular organelles)
- Can we learn to control GPCR signalling in space and time to produce novel pharmacological effects?

COMPARE PI: Meritxell Canals

Technologies used

- **BRET-based assays to interrogate receptor regulation** (arrestin and GRK recruitment, GPCR trafficking through endocytic compartments)
- **FRET-, BRET- and single fluorophore-based biosensors** to assess GPCR signalling in space and time.
- **High throughput cell signalling assays**: cAMP accumulation, calcium mobilisation, ERK and PKC activation.
- Epifluorescence, confocal, TIRF and super-resolution **microscopy**

COMPARE PI: Meritxell Canals

Main areas of research

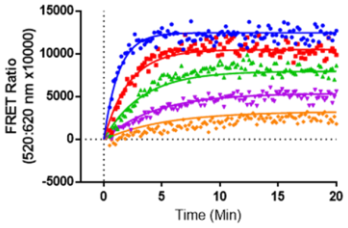
- Signalling platforms of GPCRs involved in pain transmission and modulation
- Molecular pharmacology of opioid and chemokine receptors
- Interrogation of GPCR signalling in vivo

Biological questions to be addressed

- What are the cellular mechanisms underlying the actions of different opioids?
- What is the relevance of biased agonism at the mu-opioid receptor?
- Understanding of activation mechanisms of chemokine receptors beyond the two-site two-step model.

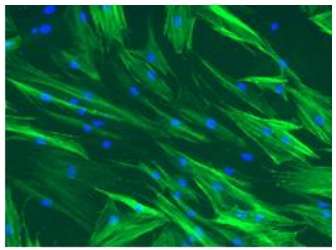
COMPARE PI: Steven Charlton

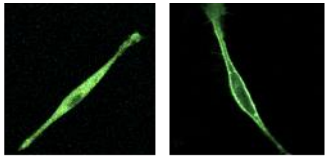
Technologies used



Fl-propranolol binding to the β_2 adrenoceptor

- TR-FRET and BRET approaches to measure the kinetics of receptor-ligand and receptor-effector binding
- Fluorescence Correlation Spectroscopy (FCS) approaches to quantify ligand concentrations in the micro-environment of cell membranes
- Biochemical assays to measure activation and kinetics of intracellular signalling molecules and transcription factors
- Phenotypic assays to measure changes in human primary lung cells related to chronic respiratory disease


Human lung fibroblast transdifferentiation


Cytoplasmic and plasma membrane targeted cAMP biosensor

- Confocal and high content imaging to monitor spatiotemporal aspects of intracellular signalling mechanisms using targeted fluorescent biosensors

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COMPARE PI: Steven Charlton

Main areas of research

- Molecular pharmacology and kinetic analysis of G protein-coupled receptors and their signalling mechanisms
- Identifying and utilising novel approaches to treat airway remodelling in chronic respiratory disease
- New target ID/validation and drug discovery

Biological questions to be addressed

- How does receptor binding and signalling in micro-compartments influence efficacy of drugs in the clinic?
- How does the spatiotemporal control of intracellular signalling molecules drive an anti-remodelling phenotype in human lung cells?

COMPARE PI: Chris Denning

Technologies used

- Human pluripotent stem cells, including embryonic and induced pluripotent stem cells.
- Transgenesis, particularly CRISPR: knockouts, knockins, substitutions, CRISPRa/i (activation / inhibition)
- Differentiation to cardiomyocytes, with growing interest in vascular cells and cardiac fibroblasts
- Phenotyping platforms, including those for electrophysiology, calcium, contraction, metabolism (Seahorse), high content imaging (Operetta)
- Robotics for drug screening

COMPARE PI: Chris Denning

Main areas of research

- Disease modelling.
- Platforms for cardiotoxicity evaluation
- Automation and interdisciplinary approaches

Biological questions to be addressed

- How can we create better models of diseases associated with defects in electrophysiology (e.g. long QT syndrome, CPVT, myotonic dystrophy), structure (e.g. myosin heavy chains, alpha-actin), survival (e.g. DMD) and signalling (e.g. b-adrenoceptors, GRK5) to gain mechanistic insight and advance diagnosis and treatment?
- How can we use compound library screening to find chemistries that improve cell function cardiomyocyte or mitigate disease status

COMPARE PI: Jonas Emsley

Technologies used

- Protein crystallography to determine 3D macromolecular structures
- Cryo-electron microscopy to study large protein macromolecular assemblies
- Surface plasmon resonance and isothermal titration calorimetry to characterise protein:ligand and protein:drug interactions
- large scale protein expression and purification using insect cells
- Enzyme kinetic assays

COMPARE PI: Jonas Emsley

Main areas of research

- Research interests include determining protein structures and studying the structure/function of complexes formed with drugs and natural ligands
- Determining structures of protein complexes formed with anti-cancer and anti-thrombosis inhibitors

Biological questions to be addressed

- What is the underlying principle determining how plasma contact proteins organise on the cell surface with respective receptors and co-factors ?
- How do these complexes contribute to coagulation and inflammation driving diverse disorders including thrombosis, cancer, hereditary angiodema and cerebrovascular diseases such as alzheimers and stroke?

COMPARE PI: Peter Fischer

Technologies used

- Application of cheminformatics, molecular modelling, and structure-based methods to the design of GPCR ligands, protein–protein and protein–oligonucleotide interaction blockers, and enzyme inhibitors.
- Traditional and fragment-based medicinal chemistry for the development of tool compounds and drug leads.
- Compound collection curation and management as a resource for screening campaigns (MCCC: *ca.* 90k chemically diverse and lead-like compounds).
- Structure elucidation (spectroscopic and spectrometric methods).

COMPARE PI: Peter Fischer

Main areas of research

- Design, synthesis, and characterisation of tool compounds and drug leads relevant to pharmacological questions in several therapeutic areas.

Biological questions to be addressed

- Affinity, selectivity, specificity, and structural basis at the molecular and atomic level of the interactions between lead- and drug-like small molecules and physiological macromolecules.
- Drug design, *e.g*. in oncology, neurodegeneration, cardiovascular and bone disease.

COMPARE PI: Caroline Gorvin

Technologies used

- Assays to measure receptor-mediated intracellular signalling
  - HTRF, AlphaScreen, intracellular calcium and luminescence based (NanoBiT, luciferase reporter) assays
- Imaging to measure receptor trafficking and signalling
  - Confocal, microfluorimetry , TIRF-M
- Whole-cell patch clamp electrophysiology and high-throughput automated patch clamp systems
- Access to large databases of cells and tissues from patients with endocrine and metabolic disease

COMPARE PI: Caroline Gorvin

Main areas of research

- Signalling and trafficking of the calcium-sensing receptor (CaSR)
- Investigating metabolic GPCRs for novel obesity therapies

Biological questions to be addressed

- How is CaSR trafficking regulated and how do human disease causing mutations affect this?
- Can GPCR heterodimerisation be exploited for novel obesity and tumour therapies?

COMPARE PI: Ian Hall

Technologies used:

- Genetic epidemiology (GWAS, sequencing etc) to identify genetic variants predisposing to respiratory disease
- Functional genetics using ex vivo human tissue, primary cell culture, animal models
- Signalling assays in human cells
- PheWAS (phenotype wide association studies) in UK biobank and related databases
- Ex vivo studies of cytokine signalling in human lung tissue and isolated cell types

COMPARE PI: Ian Hall

Main areas of research

- Identification of human genetic variants predisposing to respiratory diseases (asthma COPD etc)
- Defining mechanisms underlying genetic association signals in respiratory disease

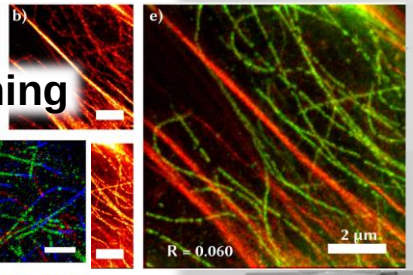
Biological questions to be addressed

- What are the key pathways driving inflammation and remodelling in airway disease such as asthma and COPD?
- How does genetic variation contribute to variability in disease risk and severity or treatment response?

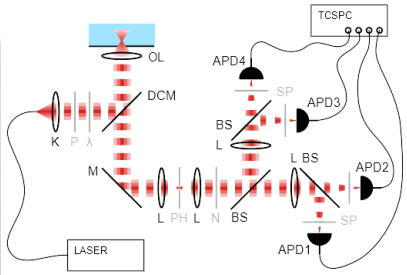
COMPARE PI: Dirk-Peter Herten

Technologies used

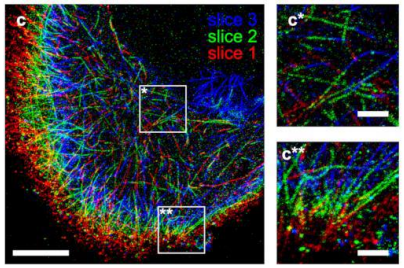
Super-Resolution Microscopy



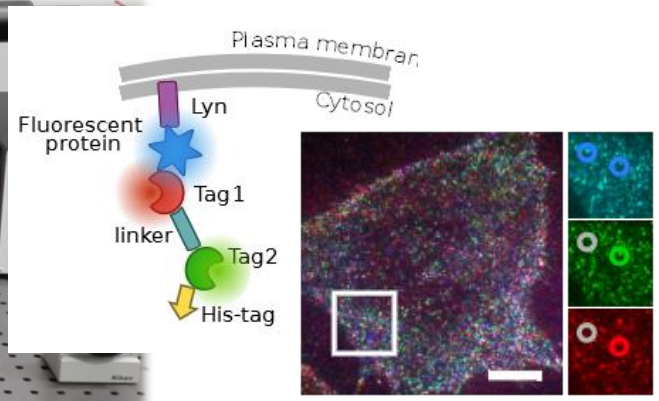
Protein counting



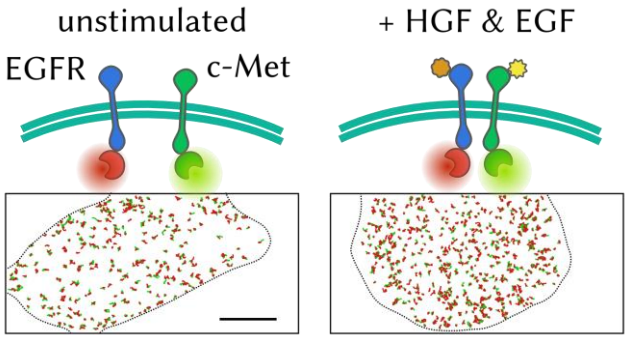
Serial Sectioning



DOL Calibration in living cells



Single-Molecule Tracking



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COMPARE PI: Dirk-Peter Herten

Main areas of research

- T-cell signalling in the context of inflammation and HIV infection
- Development of microscopy methods, e.g. for counting protein copies in cellular structures
- Fluorescent probes for super-resolution microscopy, multiplexing, studying kinetics rates of enzymatic reactions in cells)

Biological questions to be addressed

- How are T-cell microclusters organized, i.e. how many protein copies of the different adaptor proteins and kinases are there?
- How does the constitution of T-cell microclusters change upon stimulation or perturbation by forgein compounds (e.g. the HI viral protein Nef)?

COMPARE PI: Stephen Hill

Technologies used

- Development and refinement of receptor subtype-selective fluorescent ligands.
- Fluorescence Correlation Spectroscopy (FCS) approaches to quantify the speed of movement, number and brightness of single fluorescent particles (e.g. receptor complexes, ligand-receptor complexes).
- Bioluminescence Resonance Energy Transfer (BRET)-based approaches to monitor ligand-receptor binding, receptor oligomerisation and receptor-effector engagement.
- Analytical pharmacological methods to quantify affinity and efficacy.
- Assays to investigate receptor-mediated intracellular signalling.

COMPARE PI: Stephen Hill

Main areas of research

- Molecular pharmacology of G protein-coupled receptors and receptor tyrosine kinases
- Advanced imaging approaches to investigate receptor pharmacology, stoichiometry and cellular location
- Cross-talk between intracellular signalling cascadeS

Biological questions to be addressed

- How does the pharmacology of membrane receptors change as a result of their local environment and oligomeric status?
- Do these interactions differ between native cell types expressing these proteins at endogenous levels with different molecular organisations?

COMPARE PI: David Hodson

Technologies used

- High speed confocal, two photon and super-resolution imaging.
- Optogenetics and chemogenetics for conditional control of cell excitability.
- Photopharmacology including generation of ligands targeting GLP1R and KATP channels.
- Use of enyzme self-labels including SNAP and Halo tags for drug targeting.
- CRISPR-Cas9 for production of knock-in cell lines and mouse models.
- Production and testing of fluorescent peptidic ligands.

COMPARE PI: David Hodson

Main areas of research

- Molecular and cellular biology of pancreatic islet function.
- Production and validation of peptide labels for visualising class B and C GPCRs using chemical biology.
- Photopharmacology of class B and C GPCRs.

Biological questions to be addressed

- What are the mechanisms underlying glucagon, insulin and somatostatin release, with a particular focus on the role of cell subpopulations in health and disease.
- Understanding how metabolism is programmed in pancreatic beta cells and how this goes wrong during diabetes both in vitro and in vivo.

COMPARE PI: Neena Kalia

Technologies used

- Intravital microscopy for experimental imaging of tissue and organ microcirculation, specifically to quantitate circulating cell trafficking and adhesion, thrombosis, vascular leakage, functional capillary density etc.
- Laser speckle and laser Doppler to quantify changes in tissue and organ blood flow and perfusion.
- Flow cytometry to quantify vascular inflammatory damage, oxidative stress, receptor expression etc.
- Immunohistochemistry to analyse inflammatory cell infiltration, vascular oxidative stress, ligand-receptor expression etc.
- Frozen tissue, cell and immobilised protein static adhesion assays.

COMPARE PI: Neena Kalia

Main areas of research

- Microvascular damage associated with ischaemia-reperfusion injury (IRI) with a focus on the coronary microcirculation
- Optimising cellular therapy to enhance their vasculoprotective effects
- Identification of novel ligand-receptor interactions that mechanistically contribute to the microvascular damage associated with IRI.

Biological questions to be addressed

- How is the coronary microcirculation damaged by myocardial IRI?
- Do co-morbidities such as ageing and diabetes increase the susceptibility of the coronary microcirculation to IRI ?

COMPARE PI: Barrie Kellam

Technologies used

- Synthetic organic chemistry
- Chemoinformatics
- Molecular modelling (esp. GPCRs); including protein homology modelling, *in silico* docking and assessment of new ligands
- Peptide synthesis (solution & solid-phase)
- Structure elucidation (spectroscopy incl. NMR, Mass Spectrometry, Fluorimetry)
- Fluorescence Correlation Spectroscopy (FCS), Bioluminescence Resonance Energy Transfer (BRET), confocal imaging

COMPARE PI: Barrie Kellam

Main areas of research

- Synthetic Medicinal Chemistry
- Drug Discovery
- Fluorescent Ligand design, synthesis & application

Biological questions to be addressed

- New drug/probe design in numerous therapeutic areas incl. cardiovascular, pain, allergy thrombosis and cancer.
- How sub-type selective fluorescent ligands can be better designed to allow single-molecule interrogation of receptor function both *in vitro* & *in vivo.*

COMPARE PI: Rob Lane

Technologies used

- Resonance Energy Transfer (RET)-based approaches to monitor ligand-receptor binding and receptor-effector engagement.
- Analytical pharmacological methods to determine drug binding mode, affinity and efficacy.
- Assays to investigate receptor-mediated intracellular signalling.
- Development of novel chemical biology tools with which to interrogate receptor function.
- Genetic approaches with which to interrogate receptor function *in vitro* and *in vivo*.

COMPARE PI: Rob Lane

Main areas of research

- Molecular pharmacology of G protein-coupled receptors, in particular dopamine receptors
- Biased agonism and allosteric modulation of GPCRs
- Development of novel approaches to interrogate GPCR signalling *in vivo*

Biological questions to be addressed

- How does the binding mode of GPCR drugs including novel allosteric and biased GPCR ligands dictate their physiological effect?

COMPARE PI: Paula Mendes

Technologies used

- Standard and electrochemical-surface plasmon resonance spectroscopy
- Contact angle
- Ellipsometry
- X-ray photo-electron spectroscopy
- Fluorescence microscopy
- Atomic force microscopy
- Transmission electron microscopy
- Molecular Imprinting

COMPARE PI: Paula Mendes

Main areas of research

- Diagnostic molecular-based technologies
- Switchable biological surfaces
- Intracellular nanoscale sensors
- Synthetic vesicles

Biological questions to be addressed

- Accurate and early disease detection
- Materials to prevent biofouling
- On-demand sensing in the production of cells in bioreactors
- Monitor cellular processes in real-time

COMPARE PI: Robert K. Neely

Technologies used

- **Single-molecule, super-resolution (TIR) fluorescence microscopy**
  - PALM/STORM – Excellent spatial resolution
  - SOFI/SRRF – Improved temporal resolution at expense of spatial resolution
  - Expansion microscopy (ExM) – 'Easy' image acquisition with standard microscope or SPIM for rapid acquisition of 3D volumes.
  - Spectral imaging (sPAINT) – Environmental context (e.g. pH, hydrophobicity)
- **Methyltransferase (DNA/protein) enzymes** for site-specific modification/capture of targets (e.g. DNA mapping/unmethylome)
- **Nanopore sequencing** to rapidly investigate the epigenetic landscape of aberrant genomes at single-nucleotide resolution.
- **Synthetic chemistry** for the development of novel substrates which enable and facilitate the advancement of the above technologies.

COMPARE PI: Robert K. Neely

Main areas of research

- Developing and applying new strategies to study biological systems at the single-molecule level, predominantly using fluorescence microscopy.
- Enzyme-enabled DNA nanotechnology for the diagnosis of disease (oligo FISH), screening of pathogens (DNA mapping), and provision of innovative functionalities (modified DNA origami for standards, drug delivery, DNA-templated chemistry etc).

Biological questions to be addressed

- What is the spatio-temporal relationship between the suite of proteins involved in DNA repair (DSBs)?
- What is the epigenomic phenotype of a diseased (e.g. cancerous) or compromised (e.g. drugged/damaged) host?
- What 'cool' stuff can Nature be 'nudged' to do?

COMPARE PI: Natalie Poulter

Technologies used

- Single Molecule Localisation Microscopy (dSTORM)
- Structured Illumination Microscopy (SIM)
- Expansion Microscopy
- TIRF Microscopy
- Confocal Microscopy
- Live and Fixed Cell Epifluorescence Microscopy
- Platelet Function Assays

COMPARE PI: Natalie Poulter

Main areas of research

- Advanced imaging approaches to investigate platelet receptor organisation and its effect on signalling
- Understanding the role of the cytoskeleton in platelet function

Biological questions to be addressed

- How does receptor clustering affect platelet signal transduction?
- What is the role of the cytoskeleton in receptor clustering and signalling?
- How does platelet activation in suspension differ from activation via immobilised ligands

COMPARE PI: Iain Styles

Technologies used

- Image Analysis, for extracting quantitative information from image data.
- Machine learning, for understanding the relationships between the variables of a system and its observed behaviour
- Computational Topology, for understanding the intrinsic patterns in data by analysing their “shape”.
- Modelling and Simulation of Physical and Biological Systems, especially when used in conjunction with machine learning to leverage large datasets to build better models

COMPARE PI: Iain Styles

Main areas of research

- Machine learning approaches
- Understanding and quantifying static and dynamic aspects of receptor organisation from single molecule imaging data
- Methods for understanding Mass Spectrometry and Ion Mobility data

Biological questions to be addressed

- What is the role of the following in signalling?
  - Receptor oligomerisation
  - Static and dynamic organisation of receptors
  - Specific amino acids in the receptor sequence
- Structural consequences of post-translational modifications

COMPARE PI: Steve Thomas

Technologies used

- TIRF imaging
- Single molecule localisation microscopy
- Structured Illumination microscopy
- Light sheet microscopy
- Expansion microscopy
- CRISPR-Cas 9 gene editing to express fluorescently tagged proteins.
- Megakaryocyte differentiation and analysis
- Platelet functional assays

COMPARE PI: Steve Thomas

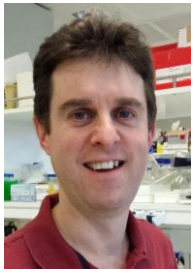
Main areas of research

- Understanding the role of the cytoskeleton in platelet and megakaryocyte function
- How does the cytoskeleton regulate membrane receptor organisation and signalling.

Biological questions to be addressed

- How does the cytoskeleton regulate invaginated membrane formation in megakaryocytes
- Organisation of the A2B receptor by the actin cytosketon
- Role of formin proteins in megakaryocyte and platelet biology

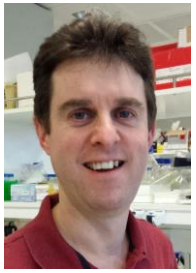
COMPARE PI: Mike Tomlinson



Technologies used

- CRISPR/Cas9 knockout of gene expression in cell lines.
- Western blotting using the Odyssey Infrared Imaging System.
- Flow cytometry to measure surface protein expression.
- Fluorescent imaging of protein dimers using bimolecular fluorescence complementation (BiFC).
- Measurement of receptor signalling using western blotting of phospho-proteins, transcriptional luciferase reporter assays and real-time PCR for transcriptionally upregulated genes.
- Measurement of membrane protein shedding using western blotting and colorimetric assays.

COMPARE PI: Mike Tomlinson



Main areas of research

- Regulation of receptor shedding (e.g. Notch, cadherins, GPVI, betacellulin, EGF) by the six TspanC8/ADAM10 scissor complexes.
- Regulation of the store-operated Ca^{2+} entry channel Orai1 by tetraspanin Tspan18.

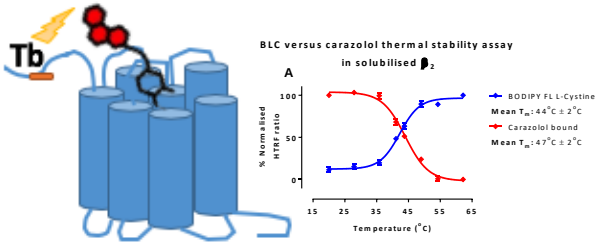
Biological questions to be addressed

- How do the six TspanC8 tetraspanins differentially regulate subcellular localisation, activation and substrate specificity of the molecular scissor ADAM10?
- How does tetraspanin Tspan18 regulate trafficking to the cell surface and activation of the Ca^{2+} channel Orai1?

COMPARE PI: Dmitry Veprintsev

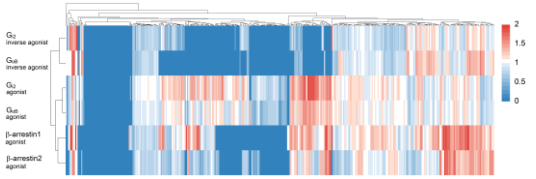
Technologies used

FRET/BRET/biophysics



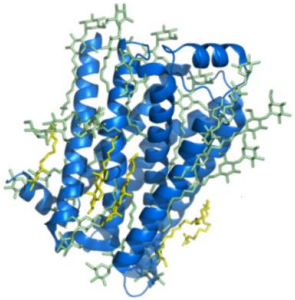
Protein purification

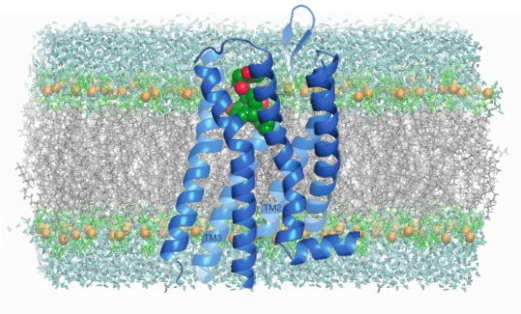
Advanced data analysis
Machine learning



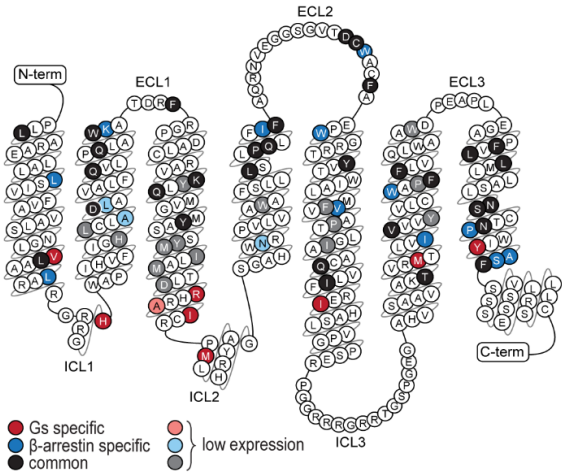
mutations
clustering

Structural bioinformatics/
Molecular dynamic simulations





Alanine scanning & HT measurements



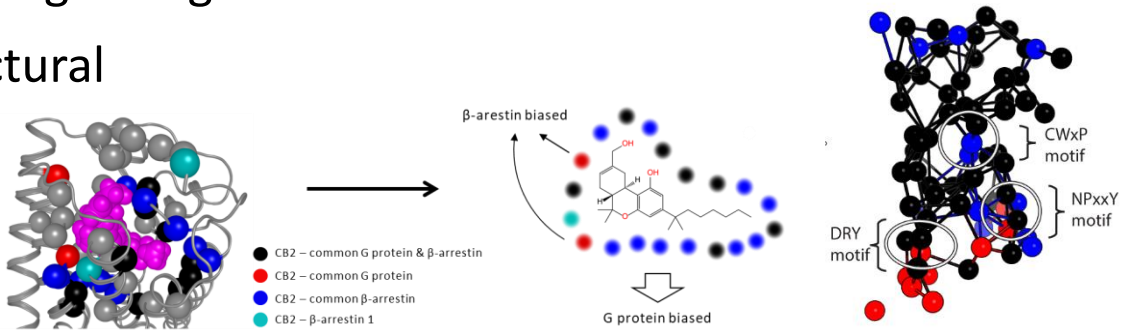
IN PARTNERSHIP:
The Universities of Birmingham and Nottingham

COMPARE
CENTRE OF MEMBRANE PROTEINS AND RECEPTORS

COMPARE PI: Dmitry Veprintsev

Main areas of research

- Molecular basis for biased signalling
- Use of AI to combine structural and functional information for biased drug design



- Shotgun approaches to study signalling bias:
  Transcriptomics & phosphoproteomics

Biological questions to be addressed

- Drug engineering: rational design of ligands with desired properties
- Deep signalling: propagation of bias through signalling cascades

IN PARTNERSHIP:
The Universities of Birmingham and Nottingham

COMPARE
CENTRE OF MEMBRANE PROTEINS AND RECEPTORS

COMPARE PI: Stephen Watson

Technologies used

- Development and refinement of receptor subtype-selective fluorescent ligands for GPVI, CLEC-2 and PEAR-1.
- Single molecule and light sheet microscopy to study clustering and movement of receptors in the cell (e.g. receptor complexes, ligand-receptor internalisation).
- Protein expression and crystalisation
- Functional assays on platelets in thrombosis, vascular integrity, wound repair, infection and inflammation focussing on liver, kidney, skin and lung
- Assays to investigate receptor-mediated intracellular signalling in platelets and cell lines.

COMPARE PI: Stephen Watson

Main areas of research

- Cell biology and pharmacology of tyrosine kinase-linked receptors (TKLRs) in platelets and megakaryocytes
- Advanced imaging and structural approaches to investigate receptor pharmacology, stoichiometry and cellular location
- Genetics of platelet bleeding disorders including GPCR mutants
- Testing of inhibitors of GPVI and CLEC-2 in thrombosis in the clinic
- Development of biologics against TKLRs

Biological questions to be addressed

- How does ligand interaction and signal strength of TKLRs change with dimerization and oligomerisation
- How does clustering of TKLRs lead to receptor activation?
- Translation of findings in mice to human

COMPARE PI: Mark Wheatley

Technologies used

A combination of molecular pharmacology, peptide chemistry and biophysical approaches to investigate G-protein-coupled receptor (GPCR) structure & function. These include:

- Detergent-free solubilisation of GPCRs in nano-scale membrane bilayer discs using polymers *e.g.* styrene maleic acid lipid particles (SMALPs).
- Radioligand binding assays.
- Second messenger assays.
- Complementary reciprocal mutation to identify ligand:receptor contacts.
- fluorescence correlation spectroscopy (FCS) to study ligand GPCR-SMALP interaction.

COMPARE PI: Mark Wheatley

Main areas of research

- Molecular pharmacology of G-protein-coupled receptors (GPCRs).
- Exploiting SMALPs for biophysical studies on GPCRs and as a platform for antibody discovery.
- Structure/function of GPCRs.

Biological questions to be addressed

- What is the molecular basis for regulation of GPCRs by ligands of different efficacy and specificity?
- How are Family B GPCRs regulated by Receptor Activity Modifying Proteins (RAMPs)?

COMPARE PI: Jeanette Woolard

Technologies used

- Doppler flowmetry and intravascular catheter implantation for haemodynamic measurements in whole living systems. This model is used to measure changes in vascular conductance in 3 distinct vascular beds, with simultaneous measurement of blood pressure and heart rate in response to administered compounds.
- Telemetric approaches to assess the long term (>28 days) changes in haemodynamic variables such as mean arterial pressure, heart rate, ECG and activity in response to administered compounds.
- Myography to investigate the effects of various compounds on smooth muscle and endothelial cell function in isolated resistance vessels.
- Bioluminescence Resonance Energy Transfer (BRET)-based approaches to monitor ligand-receptor binding, receptor oligomerisation and receptor-effector engagement.

COMPARE PI: Jeanette Woolard

Main areas of research

- Assessing the cardiovascular effects / safety of ligands which target G-protein coupled receptors (GPCRs) and receptor tyrosine kinases (RTKs).
- Identifying compounds which may prove to be possible future novel therapies for use in cardiovascular disease.

Biological questions to be addressed

- What effects do ligands which target GPCRs and RTKs have on the cardiovascular system of conscious animals and in isolated vessels?
- Can *in vitro* BRET studies involving receptor oligomerisation be used to predict physiological responses to pharmacological agents *in vivo?*