Elucidating the Molecular Basis of PPAR γ Transcriptional Plasticity by Ligand Dependent Microenvironment Mapping

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Project outline

Background: Nuclear hormone receptors (NR) have great therapeutic potential for the treatment of cardiometabolic disease. Many current NR targeting therapies, which comprise ~16% of current small molecule drugs[1], display unwanted on-target side effects. For example, the use of glitazones, PPAR γ agonists, for type 2 diabetes has been limited by weight-gain, fluid retention and cardiovascular effects. The development of selective PPAR γ ligands has been a major focus within the pharmaceutical industry. Ligand binding causes a conformational shift in PPAR γ leading to alterations in co-activator and repressor complex formation and subsequent transcriptional activity. While different ligands are known to stimulate overlapping but discrete transcriptional programs and phenotypic outcomes through PPAR γ[2], unbiased characterisation of differential co-factor binding has been limited by the technical challenges involved.

In 2020 a transformative advance in photoaffinity labelling for target identification and interactome mapping was reported. The approach uses a probe conjugated to a light-activated photocatalyst with a very short (4 nm) energy-transfer range to activate a carbene-based label. This design offers two major advantages over conventional photoaffinity labelling techniques: (1) catalytic signal amplification leads to multiple labelling events per probe, resulting in greatly enhanced signal-to-noise ratios and detection of challenging and low abundance proteins; and (2) tandem labelling of proteins within a well-defined radius – allowing interaction partners to be identified.[3] More recently cell permeable photocatalyst conjugates of small molecule probes have been described, including an example to label nuclear proteins.[4] In our lab we have already developed the methods to access these photocatalytic probes. We now propose to extend these methods to investigate the PPAR γ interactome.

Objectives: To determine the impact of diverse ligand binding on PPAR γ's microenvironment, including co-factor recruitment. To understand the dependence of the ligand-induced transcriptomic changes on the presence of specific proteins within the PPAR γ microenvironment.

Approach: Based on the extensive ligand structure-activity-relationship and crystallography data available for a range of PPAR γ ligands,[5] we will design and synthesise a small library of photoaffinity- and iridium photocatalyst-conjugated PPAR γ ligands and structure-matched controls. Following confirmation of trans-activation, probes will be applied to in vitro differentiated DFAT adipocytes[6]. Next, the samples for microenvironment mapping will be dosed with biotinylated diazirines and pulsed with blue light to initiate the labelling reaction. Through the photocatalytic labelling process, all proteins within 4nm of the photocatalyst-conjugated PPAR γ ligands will be labelled by the biotinylated diazirine, allowing pulldown and analysis. Labelled proteins will be enriched and analysed by

proteomics with samples for transcriptomic analysis processed in parallel. Parallel experiments on in vitro differentiated DFAT adipocytes with inducible PPAR γ KO will be used to ensure the specificity of identified proteins and to distinguish 'off-target' labelling from genuine interactome labelling. Ligands will be clustered based on proteomic and transcriptomic signatures to identify proteins correlating with transcriptional changes. The necessity of identified proteins in mediating the ligand induced transcriptomic changes will be determined by assessing ligand induced transcriptomic changes in cells with the identified proteins knocked out.

Impact: This work will provide fundamental insight into the regulation of gene transcription by PPAR γ. Such insight could inform the development of PPAR γ-based therapeutics with desired properties and enable alternative approaches such as inhibiting interactions between PPAR γ and specific co-factors. Additionally, this project would provide proof of concept for a novel application of this state-of-the-art technology that could be applied to other receptors or to perform target deconvolution of novel bioactive small molecules or peptides.

References: [1]Santos et al., *Nat. Rev. Drug Discov.* **2017**, *16*, 19-34. [2]Liu et al., *J Med. Chem.* **2011**, 54, 8541–8554 [3]Geri et al., *Science* **2020**, *367*, 1091–1097; [4]Trowbridge et al. *BioRxiv*, DOI: 10.1101/2021.08.02.454797. [5]For example see (a) Colca *et al.*, *Am J Physiol Endocrinol Metab* **2004**, *286*, E252–E260; (b) Hughes et al., *Nat Commun* **2014**, *5*, 357. [6]Loh et al., *Nat Commun* **2020**, *11*, 2797.

Contributions of Oxford and NNRCO supervisors:

Chemistry/Pharmacology: Photoaffinity and photcatalytic probe design using in silico modelling. Probe synthesis and preliminary characterisation. PPAR gamma antagonism/agonism assessment. Probe labelling workflow optimisation.

NNRCO: Adipocyte cell culture including genetic modifications, transcriptomics, proteomics

It would be expected to recruit a fellow with experience in chemical biology, having worked at the interface of ligand design and synthesis, biochemistry and cell biology. It would be expected that most of the planned activities, including those carried out at NNRCO, would be conducted by the fellow supported by existing in-house expertise. In the last 5-10 years there has been increasing emphasis (notably in the UK, US and Europe) on training early career researchers at the interface of chemistry and biology and we are confident we would be able to recruit a high calibre individual with the requisite skills in chemistry, and molecular and cell biology.

Supervisor's recent relevant publications (5 max per supervisor):

Prof Angela Russell
1. Wilkinson, I.V.L.; Perkins, K.J.; Dugdale, H.; Moir. L.; Vuorinen, A.; Chatzopoulou, M.; Squire, S. E.; Monecke, S.; Lomow, A.; Geese, M.; Charles, P.D.; Burch, P.; Tinsley, J.M.; Wynne, G.M.; Davies, S.G.; Wilson, F.X.; Rastinejad, F.; Mohammed, S.; Davies, K.E. **Russell, A.J.*** Chemical Proteomics and Phenotypic Profiling Identifies the Aryl Hydrocarbon Receptor as a Molecular Target of the Utrophin Modulator Ezutromid. Angew. Chem. Int. Ed. 2020; DOI: 10.1002/anie.201912392.

2. Josa-Culleré L, Madden KS, Cogswell TJ, Jackson TR, Carter TS, Zhang D, Trevitt G, Davies SG, Vyas P, Wynne GM, Milne TM* and **Russell AJ*** A Phenotypic Screen Identifies a Compound Series That Induces Differentiation of Acute Myeloid Leukemia Cells In Vitro and Shows Antitumor Effects In Vivo. J. Med. Chem. 2021, 64, 15608–15628. DOI: 10.1021/acs.jmedchem.1c00574.

3. Quevedo, C.E., Cruz-Migoni, A., Bery, N., Miller, A.,Tanaka, T., Petch, D., Bataille, C.J.R., Lee, L.Y.W., Fallon, P.S., Tulmin, H., Ehebauer, M.T., Fernandez-Fuentes, N., **Russell, A.J.**, Carr, S.B., Phillips, S.E.V., Rabbitts, T.H. Small molecule inhibitors of RAS-effector protein interactions derived using an intracellular antibody fragment, Nature Commun., 2018, 9. DOI: 10.1038/s41467-018-05707-2.

4. Stefaniak, J., Lewis, A.M., Conole, D., Galan, S.R.G., Bataille, C.J.R., Wynne, G.M., Castaldi, M.P., Lundbäck, T., **Russell, A.J.*** and Huber, K.V.M.* Chemical Instability and Promiscuity of Arylmethylidenepyrazolinone-Based MDMX Inhibitors. ACS Chem. Biol. 2018, 13, 10, 2849–2854.

5. Kerr, A.G.; Tam, L.C.S.; Hale, A.B.; Cioroch, M.; Douglas, G.; Agkatsev, S.; Hibbitt, O.; Mason, J.; Holt-Martyn, J.; Bataille, C.J.R.; Wynne, G.M.; Channon, K.M.; **Russell, A.J.***; Wade-Martins, R. A genomic DNA reporter screen identifies squalene synthase inhibitors which act cooperatively with statins to upregulate the low-density lipoprotein receptor. J. Pharmacol. Exp. Ther. 2017, 361, 417–428.

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Dr Max Ruby

1. **Ruby MA**, Riedl I, Massart J, Åhiln M, Zierath JR. Skeletal Muscle Protein Kinase N2 regulates AMP-Kinase and Insulin Responsiveness. Am J Physiol Endocrinol Metabl. 313:E483-E491, 2017.

2. **Ruby MA**, Massart J, Hunderosse DM, Schönke M, Correia JC, Louie SM, Ruas JL, Näslund E, Nomura DK, Zierath JR. Human Carboxylesterase 2 Reverses Obesity-Induced Diacylglycerol Accumulation and Glucose Intolerance. Cell Rep. 18:636-646, 2017.

3. Kirchner H, Sinha I, Gao H, **Ruby MA**, Schönke M, Lindvall JM, Barres R, Krook A, Näslund E, Dahlman-Wright K, Zierath JR. Altered DNA methylation of glycolytic and lipogenic genes in liver from obese and type 2 diabetic patients. Mol Metab. 5:171-183, 2016.

4. **Ruby MA**, Goldenson B, Orasanu G, Johnston TP, Plutzky J, Krauss RM. VLDL hydrolysis by LpL activates PPAR-α through generation of unbound fatty acids. J Lipid Res 51:2275-2281, 2010.

5. **Ruby MA^**, Nomura DK^, Hudak CS, Mangravite LM, Chiu S, Casida JE, Krauss RM. Overactive endocannabinoid signaling impairs apolipoprotein E-mediated clearance of triglyceride-rich lipoproteins. Proc Natl Acad Sci USA 105:14561-14566, 2008 **^contributed equally to this work**