Name	LOUISE WALPORT
Position	Physical Science Group Leader (Imperial)
Year joined *(Crick or founder institute)*	2018

Career History

2009- 2014 - D.Phil Lady Margaret Hall, University of Oxford funded by BBSRC studentship
2014- 2015 - PDRA Department of Chemistry, University of Oxford
2015- 2018 - Marie Skłodowska-Curie Global Fellow, University of Tokyo and University of Oxford,

Major Awards, Honours and Prizes

Membership of external committees, editorial boards, review panels, SABs etc

Member of the Royal Society of Chemistry Protein and Peptide Science Group Committee: November 2019 onwards

Lab Name	***Protein-Protein Interaction Laboratory***

Research programme and achievements

My lab was established in October 2018. We apply a state-of-art cyclic peptide discovery system, known as the RaPID system, to develop chemical probes with which we and others can manipulate a range of biological systems. We have achieved our initial goal of establishing the RaPID screening technology in house. Since then, each lab member has begun working on individual but complementary projects towards our shared lab vision.

Work in the group builds on my background in chemical probe discovery for epigenetic targets, assay development and enzymology. Prior to joining the Crick, I demonstrated that arginine methylation could be removed by a family of non-haem Fe(II) enzymes, settling a long-standing question in the field as to whether this modification was a reversible post-translational modification (PTM). My lab has maintained an interest in arginine PTMs and now focuses on the peptidyl arginine deiminases (PADIs) which convert arginine to citrulline (see below). More recently, in a study profiling the diversity of cyclic peptides found within encoded libraries (in collaboration with the Mackay Lab (University of Sydney)) we have revealed that encoded peptide libraries contain members with many different structural elements that can bind to similar proteins in surprisingly

diverse ways. This work provides an experimental explanation for the widely accepted view that peptide library approaches can be used to identify potent and selective protein binders of almost any target of choice.

On the technology development side, my lab now seeks to extend the chemical functionalities that can be included in peptides within DNA-encoded libraries to further expand their applicability. This currently includes incorporating photoswitchable moieties to produce tools that can be switched “on” and “off”, reactive chemical functionalities to produce covalent protein binders and non-canonical amino acids that allow direct visualisation of peptides in cells or enhance their proteolytic stability. We are also exploring the possibility of developing more tailored methods to identify peptide tools with defined functionalities, such as protein activation. We use the RaPID system as a model but aim to develop strategies that could be applied more widely to other DNA encoded drug discovery platforms.

On the biomedical application side, we are using the peptide tools we produce, combined with other more classical biochemical and molecular biology techniques, to rigorously explore the biological significance of protein citrullination in health and disease. The long-term aim of this work is to connect the biochemical effects of protein citrullination with animal physiology, informing on how the chemistry of (methylated)guanidino and citrulline groups relates to biological function; a field in which there has been relatively little work compared to many other PTMs.

We have produced peptide inhibitors and first-in-class activators of the nuclear member of the PADI family, PADI4. These highly selective peptides provide us with tools to both increase and decrease PADI4 activity in a precise and specific manner. We are currently applying these in cell-based systems to better understand its cellular regulation. We also have a particular interest in the fifth member of the PADI family, PADI6. PADI6 is essential for early embryogenesis, with mutations and deletions resulting in female infertility. However, despite this profound phenotype, the precise molecular mechanisms of PADI6 biological activity remain to be elucidated. We are currently combining our own *in vitro* expertise, with *in/ex vivo* approaches used in the Turner and Niakin labs to dissect the molecular mechanisms behind this phenotype. This approach has been enabled by my location at the Crick. In the longer term I anticipate exploring the use of peptide tools to further manipulate PADI6 activity and understand whether it could be therapeutically targeted.

In addition, we also work with others at the Crick to produce tools to address their research questions. We have established collaborations with several other Crick researchers, including Simon Boulton and Jernej Ule, to produce tools that could be used to explore their biology. Over the remainder of my time at the Crick I hope to be able to extend these and other collaborations. I anticipate maintaining these well beyond my return to Imperial.

Research outputs

Karishma Patel,† Louise J. Walport,†* James L. Walshe, Paul D. Solomon, Jason K. K. Low, Daniel H. Tran, Kevork S. Mouradian, Ana P. G. Silva, Lorna Wilkinson-White, Alexander Norman, Charlotte Franck, Jacqueline M. Matthews, J. Mitchell Guss, Richard J. Payne, Toby Passioura, Hiroaki Suga,* Joel P. Mackay* (†these authors contributed equally to this publication) (*co-corresponding authors). (2020) *Cyclic*

peptides can engage a single binding pocket through highly divergent modes. **PNAS 117 (43) 26728 – 26738. DOI: 10.1073/pnas.2003086117**
This publication highlights the power of the RaPID system to produce potent and highly selective inhibitors. Despite their widespread application, the inherent structural diversity in encoded cyclic peptide libraries has been little explored. Here, through screening against several highly conserved proteins with a single library, we revealed unprecedented structural and functional diversity in peptide hits. This provides an experimental explanation for the wide applicability of these libraries.

Walport LJ, Hopkinson RJ, Chowdhury R, Schiller R, Ge W, Kawamura A, Schofield CJ. (2016) ***Arginine demethylation is catalysed by a subset of JmjC histone lysine demethylases.*** **Nature Communications 7, 11974. DOI: 10.1038/ncomms11974**
In this publication we demonstrate that a family of enzymes that are known to catalyse lysine demethylation of histones can also catalyse the demethylation of arginine residues. This addressed a longstanding question in the field as to whether this PTM was reversible. The publication highlights my use of multiple approaches (here including work with peptides, *in vitro* kinetics, x-ray crystallography and cell-based experiments) to dissect enzymatic function, an approach we continue to take in the lab.

Kawamura A, Münzel M, Kojima T, Yapp C, Bhushan B, Goto Y, Tumber A, Katoh T, King ONF, Passioura T, Walport LJ, Hatch SB, Madden S, Müller S, Brennan PE, Chowdhury R, Hopkinson, RJ, Suga H, Schofield CJ. (2017) ***Highly selective inhibition of histone demethylases by de novo macrocyclic peptides*****. Nature Communications 8, 14773. DOI: 10.1038/ncomms14773**
This publication demonstrates how the RaPID system can be used to develop extremely selective compounds against conserved enzyme families. In particular it highlights how highly specific cyclic peptide tools can be optimised and applied to modulating an epigenetic target in a cellular context. We are applying similar strategies to new peptides identified in my lab.

Hsu, KF, Wilkins SE, Hopkinson RJ, Sekirnik R, Flashman E, Kawamura A, McCullagh JSO, Walport LJ*, Schofield CJ* (*co-corresponding authors). (2021) ***Hypoxia and hypoxia mimetics differentially modulate histone post-translational*****.**
Epigenetics 16(1):14-27. DOI: 10.1080/15592294.2020.1786305
In this paper we profile the effects of hypoxia mimetic drugs on histone post-translational modifications and compare this with hypoxia itself. It highlights our approach to using chemical tools responsibly. Used appropriately synthetic drugs can be powerful tools to modulate biology, but they must always be carefully profiled and used with caution to avoid data misinterpretation.