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| **Name** | KATRIN RITTINGER |
| **Position** | Senior Group Leader<br>Assistant Research Director |
| **Year joined *(Crick or founder institute)*** | 1996 |

**Career History**

1991-1994: PhD in Chemistry, Max-Planck Institute for Medical Research & Heidelberg University, Germany
1995: Post-doctoral Research Associate, Max-Planck Institute for Molecular Physiology, Dortmund, Germany
1996-2000: Post-doctoral Research Associate, MRC-National Institute for Medical Research, London, UK (EMBO LT and Marie Curie Fellowships)
2000-2006: Tenure-track Group leader, MRC-NIMR, London, UK
2006-2015: Group Leader, MRC-NIMR, London, UK
2015 -present: Senior Group Leader, Francis Crick Institute, London

**Major Awards, Honours and Prizes**

2019: Elected Member of EMBO

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

**Editorial Bboards:**
2007-present: Biochemical Journal Editorial Board
2014-2017: Royal Society Open Science Subject Editor

**Review panels & SABs etc**
2017-present: GSK-Crick LinkLabs Joint Steering committee
2015-present: CRUK New Investigator Panel Member
2016-present: iNEXT/Instruct Review Panel Member
2019: Norwegian Research Council Panel Member
Since 2020: SAB member for UBIMOTIF (MSC-ITN network)

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| **Lab Name** | ***Molecular Structure of Cell Signalling Laboratory*** |

**Research programme and achievements**

Protein ubiquitination is a highly versatile post-translational modification that is used in the regulation of virtually all cellular processes, from the maintenance of protein homeostasis to DNA damage responses, cell cycle regulation and immune signalling. E3 ubiquitin ligases, the family of enzymes catalysing the last step of the three-step ubiquitination cascade, select the substrate to be modified, and in some cases also determine the topology of the polyubiquitin chain formed, and thus the physiological outcome. Alterations in the ubiquitin system have been linked to many diseases, including autoimmune and inflammatory disorders, cancer and neurodegenerative diseases. Hence there is immense interest in developing therapeutics that target the ubiquitin system. However, progress towards this endeavour has been slow, and there is a clear need for a more detailed understanding of the structures, molecular mechanisms and regulatory elements of the enzymes involved to enable a more successful drug discovery process.

Research in my group is aimed at providing a molecular understanding of the mechanisms underlying the activity and regulation of enzymes of the ubiquitination cascade, especially E3 ubiquitin ligases, thereby contributing to efforts to explore the ubiquitin system as a drug target. We are employing a broad experimental approach to characterise the function of these E3 ligases on a structural and mechanistic level and collaborate with cell biologists and immunologists to test our working models in a biological context, and with chemists to develop chemical tools to specifically target ubiquitination enzymes.

During this quinquennium we have made important progress towards understanding the mechanism and activity of members of the TRIM family of E3 ligases, many of which regulate innate immune responses. Self-association into higher order assemblies is a key functional property that directs the catalytic activity of this protein family and our work has provided important new insight into the link between oligomeric state and ubiquitination activity. Furthermore, our studies have provided a molecular explanation for the mechanism used by an influenza protein to interfere with TRIM25 function and thereby suppress a host immune response.

In parallel, we have been studying the mechanism of ubiquitin transfer employed by RBR ligases, an E3 subfamily with a catalytic module comprising three subdomains separated by flexible linkers. These subdomains have to work in a coordinated manner to recognise the ubiquitin-loaded E2 and transfer ubiquitin onto a catalytic cysteine within the E3, before its final transfer onto the substrate. Combining structural, biophysical and biochemical techniques we have provided important insight into the mechanism regulating the activity of RBR ligases, especially of the multi—protein E3 LUBAC which is a key regulator of immune and inflammatory signalling, and apoptosis.

We will extend our work on TRIM ligases to cover members from each of the 11 sub-classes of this protein family to identify possible common mechanistic principles that guide the activity of this family. In parallel, we will explore the biological functions of specific subgroups to gain insight into how their E3 ligase activity relates to other biological processes that have been described for specific family members.

At present, no inhibitors or chemical probes specific for TRIM family members exist. We will use chemical biology approaches including fragment-based covalent ligand screening to test if the substrate binding domains of TRIMs could be targeted for chemical probe development. Similarly, in collaboration with GSK/LinkLabs we are extending and improving our fragment-based covalent ligand library to target other E3 ligases containing catalytic cysteine residues, including the RBR ligases discussed above, and bacterial E3 ligases of the NEL family, which are secreted effector proteins. To

develop this technology further and allow screening of covalent libraries in a cellular context without the need to purify individual proteins, we have extended our LinkLabs collaboration to include Bram Snijders from the Proteomics STP to develop fast and high-throughput chemoproteomic pipelines.

In addition to the work on protein ubiquitination, we have established a collaboration with Teresa Thurston from Imperial College London, to complement the cellular work from her group on *Salmonella* effector proteins with structural and mechanistic studies, in order to provide a detailed understanding of how bacterial effector proteins manipulate innate immune signalling.

## Research outputs

**Tsai YI, Johansson H, Dixon D, Martin S, Chung CW, Clarkson J, House D, Rittinger K. (2020) *Single domain antibodies as crystallization chaperones to enable structure-based inhibitor development for RBR E3 ubiquitin ligases*. Cell Chem Biol. 27(1):83-93.e9. DOI: 10.1016/j.chembiol.2019.11.007**
In collaboration with GSK and the Crick-GSK LInkLabs we selected single-domain antibodies (dAbs) based on a human scaffold that recognise the catalytic domain of HOIP, a subunit of the multi-component E3 ligase LUBAC. We used these dAbs to interrogate the ubiquitin transfer mechanism of HOIP, and as crystallisation chaperones to crystallise a HOIP RBR/dAb complex. This complex now serves as a robust platform for soaking of ligands that target the active site cysteine of HOIP, thereby providing easy access to structure-based ligand design for this important class of E3 ligases.

**Johansson H, Isabella Tsai YC, Fantom K, Chung CW, Kumper S, Martino L, Thomas DA, Eberl HC, Muelbaier M, House D, Rittinger K (2019) *Fragment-Based Covalent Ligand Screening Enables Rapid Discovery of Inhibitors for the RBR E3 Ubiquitin Ligase HOIP*. J Am Chem Soc 141: 2703-2712. DOI: 10.1021/jacs.8b13193**
Protein ubiquitination is a key regulatory mechanism and E3 ubiquitin ligases are the key mediators of ubiquitination providing specificity to the process. In this study we describe the application of fragment-based covalent ligand screening to target the active site of an E3 ubiquitin ligase (HOIP) for which previously no specific inhibitors were known. Combining chemical biology, X-ray crystallography, chemoproteomics and cell biology we were able to identify a covalent binder for HOIP that now forms the basis for further inhibitor development. This study illustrates more generally the power of fragment-based covalent ligand screening to identify lead compounds against challenging targets.

**Koliopoulos MG, Lethier M, van der Veen AG, Haubrich K, Hennig J, Kowalinski E, Stevens RV, Martin SR, Reis ESC, Cusack S, Rittinger K (2018) *Molecular mechanism of influenza A NS1-mediated TRIM25 recognition and inhibition*. Nat Commun 9: 1820. 10.1038/s41467-018-04214-8**
Modification with K63-linked poly-ubiquitin chains of RIG-I, a viral RNA sensor that induces type I IFN production in response to viral infection, is crucial for activation of the RIG-I/MAVS signalling pathway. These chains are synthesised by the E3 ubiquitin ligase TRIM25, which is targeted by influenza A virus non-structural protein 1 (NS1) to prevent an efficient host immune response. In this study we provide molecular insight into the mechanism by which NS1 interferes with the correct positioning of the substrate-binding

PRYSPRY domain of TRIM25 to suppresses RIG-I ubiquitination and hence downstream signalling.

**Esposito D, Gunster RA, Martino L, El Omari K, Wagner A, Thurston TLM, Rittinger K (2018) *Structural basis for the glycosyltransferase activity of the Salmonella effector SseK3*. J Biol Chem 293: 5064-5078. DOI: 10.1074/jbc.RA118.001796**
A key part of *Salmonella* pathogenesis is the delivery of virulence proteins (effectors) into the host cell to interfere with host immune responses. SseK3 is a glycosyltransferase that transfers an *N*-acetylglucosamine (GlcNAc) moiety onto a target arginine of host proteins, thereby modulating host cell function. In this study we describe the first detailed structural and functional characterisation of SseK3, which provided important insight into its enzymatic mechanism and substrate selection.

**Koliopoulos MG, Esposito D, Christodoulou E, Taylor IA, Rittinger K (2016) *Functional role of TRIM E3 ligase oligomerization and regulation of catalytic activity*. *EMBO J* 35: 1204-18. DOI: 10.15252/embj.201593741**
This study presents the first quantitative analysis of the link between the oligomeric state and catalytic activity of TRIM ligases, an important class of E3 ligases that play crucial roles in the regulation of innate immune repsonses, amongst other cellular functions. Our detailed structural and biochemical analysis allowed us to propose models for the architecture of TRIM25 and TRIM32 that differ in the manner in which dimerisation of the catalytic RING domain, which is absolutely required for activity, is achieved. This study now forms the basis for further work interrogating the function of this protein family.