Name	STEVE GAMBLIN
Position	Senior Group Leader Director of Scientific Platforms
Year joined *(Crick or founder institute)*	1994

Career History

1983 - 1988	Ph.D, Department of Biochemistry, University of Bristol, UK
1988 - 1990	Postdoctoral Research Associate, Biochemistry, University of Bristol, UK
1990 - 1994	Howard Hughes Postdoctoral Research Fellow, Department of Biochemistry and Molecular Biology, Harvard University, USA
2005 - 2015	Group Leader, Division of Protein Structure, NIMR
2005 - 2015	Joint Head of Division of Molecular Structure, NIMR
2010 - 2015	Director of Research, NIMR
2015 - 2018	Group Leader & Director Science Operations, Francis Crick Institute
2018 –	Group Leader & Director of Scientific Platforms Francis Crick Institute

Major Awards, Honours and Prizes

2012	Research prize - Feldberg Foundation
2011	Elected Fellow of the Royal Society
2008	Elected Fellow of Academy of Medical Sciences
2007	Elected EMBO Member

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

Lab Name	***Structural Biology of Disease Processes Laboratory***

Research programme and achievements

My lab investigates the mechanisms of the cell's protein machinery using structural biology and biophysics guided methodologies. We have three main interests. Firstly, chromatin modification complexes that regulate transcription and have been implicated in cancer. Secondly, viral proteins that facilitate infectivity. In this area historically we have focused on influenza but more recently we have used our experience in these systems to investigate the analogous mechanisms in coronavirus. Finally, we have a longstanding interest in the cellular energy regulator AMP-activated Protein Kinase (AMPK) whose misregulation is linked to diabetes. Our underlying philosophy is that the discovery of the molecular mechanism of fundamental processes will reveal insights into the disease process that will ultimately drive the development of novel therapeutic approaches.

During this quinquennium we completed a long-term project aimed at determining the mechanism that drives allosteric activation of the polycomb repressive complex (PRC2) and leads to spreading of the histone H3K27 methyl silencing mark to generate heterochromatin. Principally this involved the determination of the crystal structure of the catalytic core of the PRC2 complex, bound to both stimulating product and substrate peptides (Justin et al 2016). The structure revealed how the three core subunits interlock to form the catalytic core, and how product binding to a non-catalytic subunit stabilises a series of elements that ultimately promote methylation at the active site. We were then able to exploit the knowledge gained through this initial structure to follow this work directly with a structural investigation of PRC2 blocked with an inhibitor compound, developed by colleagues in pharma (Constellation Pharmaceuticals) (Vaswani et al 2016). This revealed how the inhibitor binds all three subunits locking the complex into an inactive conformation and prevents the binding of cofactor. This structure is being used for further drug development aimed at cancer treatment.

We have had a productive history of using X-Ray crystallography combined with biophysics to investigate the evolution of the major influenza protein, haemagglutinin (HA). This has been used to explain how emerging strains of seasonal influenza have modified through mutation of strains in non-human hosts to bind to human receptors. This work is a close collaboration with John Skehel and our colleagues at the World Influenza centre at the Crick. In the current quinquennium we have been able to exploit the recent dramatic advances in resolution of structural Cryo EM technology to further our understanding of the dynamic process of invasion mediated by HA. A high resolution (3.3 Å for the ectodomains) EM structure of full-length HA revealed for the first-time details of how HA is anchored in the virus membrane and the significance of the flexible linker that connects the membrane anchor to the ectodomain (Benton et al 2018). In further work we showed how binding of a FAB antibody fragment to this region disrupts flexibility and may therefore reduce infectivity. Developing these techniques we have more recently been able to determine structures of intermediates in the endocytosis pathway. As part of the process of cell invasion by the virus the HA proteins undergo a large conformational transformation that ultimately results in fusion of the virus particle to the cell membrane (Benton et al 2020). Using a novel approach, we were able to follow the structural changes to HA induced by incubating the protein at fusion pH, and revealed how the HA1 domain dilates and extends before folding back into the post fusion conformation.

Finally, more recently, in response to the covid crisis we were able to apply our experience in the structural biology of viral infectivity to investigate the changes to the SARS-Cov2 spike protein and its binding to ACE receptors (Wrobel et al 2020). We have shown the importance of the acquired furin cleavage site in enabling the spike protein to adopt a conformation that is favourable to bonding the human receptor.

Research outputs

Justin, N. et al. (2016) *Structural basis of oncogenic histone H3K27M inhibition of human polycomb repressive complex 2*. Nat Commun 7, 11316. DOI: 10.1038/ncomms11316

The PRC2 complex is the major epigenetic driver of programmed transcriptional repression in the cell and its misregulation is associated with development defects and genomic instability. It is a major target for cancer therapeutics. In this paper we described the crystal structure of the multi-protein catalytic core of the PRC2 complex. This analysis provided a description for the molecular basis of allosteric regulation of the EZH2 methyltransferase

subunit by its product. Further, the structure contains an oncogenic histone peptide and revealed the mechanism underlying the stalling of the spread of the H3K27 silencing mark in glioma.

Benton DJ, Nans A, Calder LJ, Turner J, Neu U, Lin YP, Ketelaars E, Kallewaard NL, Corti D, Lanzavecchia A, Gamblin SJ, Rosenthal PB, Skehel JJ. (2018) *Influenza hemagglutinin membrane anchor*. Proc Natl Acad Sci USA 115(40):10112-10117. DOI: 10.1073/pnas.1810927115
My lab has a strong record of publications describing the emerging properties of the influenza haemagglutinin (HA) surface protein and its role in infection. In this publication we were able to present a description of the high resolution cryo EM structure of the full-length HA. This structure represented a major development because, for the first time, it includes not just the ectodomain, but also the membrane anchor. This reveals how the HA protein is embedded in the virus membrane and the significance of the flexible linker that connects the membrane anchor to the ectodomain.

Benton DJ, Gamblin SJ, Rosenthal PB, Skehel JJ. (2020) *Structural transitions in influenza haemagglutinin at membrane fusion pH*. Nature 583(7814):150-153. DOI: 10.1038/s41586-020-2333-6.
In order to understand the function of HA in influenza infectivity it is necessary to understand the mechanism of endocytosis. It has previously been established that endocytosis involves a large conformational rearrangement of the HA protein that can be triggered by a change in pH, revealed by structures of initial and final states. In this paper we adapted the cryogenic EM technique in order to trap a series of intermediate HA structures in the pathway. This provided the most complete picture to date of the structural rearrangements that occur in HA during the endocytosis process.

Wrobel AG, Benton DJ, Xu P, Roustan C, Martin SR, Rosenthal PB, Skehel JJ, Gamblin SJ. (2020) *SARS-CoV-2 and bat RaTG13 spike glycoprotein structures inform on virus evolution and furin-cleavage effects*. Nat Struct Mol Biol. 27(8):763-767. DOI: 10.1038/s41594-020-0468-7
We have been able to apply the knowledge we have gained from our work on the infectivity of the influenza virus to the challenge presented by the recent SARS-CoV-2 virus outbreak. In this paper we present high resolution cryo EM structures of the SARS-CoV-2 and bat RaTG13 spike glycoproteins. We describe from a structural perspective the significant differences between the strains. We draw particular attention to the addition of a furin cleavage site into the human virus spike protein. We discuss its potential role in infectivity and on the evolution of this virulent strain.

Benton, D.J., Wrobel, A.G., Xu, P. Roustan, C., Martin, S.R., Rosenthal, P.B. Skehel, J.J. & Gamblin, S.J. (2020) *Receptor binding and priming of the spike protein of SARS-CoV-2 for membrane fusion*. Nature 588, 327–330. DOI: 10.1038/s41586-020-2772-0
Here we describe the conformational changes that the SARS-Cov2 spike protein undergoes in binding to the human ACE2 receptor. This represents the initial stages of the mechanism of cell invasion by the virus particle during infection. We show a series of ten cryoEM reconstructions of the spike protein binding to ACE2 through its receptor binding domain (RBD), ranging from a closed unbound spike ectodomain trimer to the fully open conformation with each RBD in the trimer bound to an ACE2 receptor. Binding to ACE2 releases the so-called fusion peptide segment and promotes membrane fusion leading to cell invasion.