STEVEN HARDWICK

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Education and Research Experience

2007- **Research Associate**
Department of Biochemistry, University of Cambridge
Group Leader: Prof. B. F. Luisi

Synopsis: The main focus of research in our group is on a bacterial multi-protein RNA processing machine termed the RNA-degradosome. The full degradosome from *Escherichia coli*, and the individual protein components of the complex, Endoribonuclease E (RNase E), a DEAD-box RNA helicase (RhlB), the glycolytic enzyme Enolase and the phosphorolytic exoribonuclease PNPase have been studied by many biochemical and biophysical techniques including isothermal titration calorimetry, surface plasmon resonance, and primarily X-ray crystallography. I have been primarily studying an RNA degradosome complex in *Caulobacter crescentus*, and have identified by co-immunoprecipitation a complex composed minimally of RNase E, a DEAD-box RNA helicase similar to the *E. coli* RhlB, PNPase and the TCA cycle enzyme Aconitase. I have also solved the crystal structure of the *Caulobacter crescentus* PNPase bound to endogenous RNA, revealing asymmetry and potential allostery in the mechanism of RNA binding and processing. The current focus of my research is to use *C. crescentus* as a model of bacterial cell development, and determine whether the degradosome complex is the target of cell cycle dependent regulation.

I am additionally involved with several other projects within the lab studying various proteins involved in regulating RNA metabolism in bacteria. These include the RNA chaperone/binding proteins ProQ and RapZ from E. coli, and a bacteriophage encoded inhibitor of RNase E we have termed Dip (Degradosome interacting protein).

2003-2007 **PhD in Structural Biology – Newcastle University, UK.**
Title: Structural studies of regulators of sigma B in gram positive bacteria.
Supervisor: Prof. R. J. Lewis

Synopsis: When the bacteria B. subtilis and S. aureus encounter environmental or physical stresses the alternative sigma factor, σB directs RNA polymerase to the promoters of over 200 genes encoding the general stress proteins. The activity of σB is controlled by a set of proteins termed regulators of sigma B (Rsb).
Throughout my PhD I studied some of the interactions between the Rsb proteins, predominantly the activation of the type 2C protein phosphatase RsbU via an interaction with its activator protein RsbT.
The B. subtilis protein RsbU is composed of a C-terminal type 2C phosphatase domain and an N-terminal domain presumed to be involved in binding to the phophatase activator protein RsbT, an interaction that leads to a 20 fold increases in phosphatase activity towards phosphorylated RsbV (2). I determined experimentally that the N-terminal domain of RsbU is indeed an RsbT binding domain, and furthermore I discovered four residues on the surface of N-RsbU crucial for the binding to RsbT.

2000-2003 **Newcastle University, UK.**
BSc Hons. Medical Microbiology (Grade 2.1)
Including research project: Identification of the cadmium binding site of CtpR in Mycobacteria in vivo
Supervisor: Dr. J. S. Cavet.

Teaching Experience:

2014-2017 **Session chair – PartIII research symposium**
Lead questions/discussion during "Structural Biology and Drug Discovery" session at Masters students research presentation session

2013- **Honorary Post-doctoral teaching fellow**
Participant in 2nd and 3rd year undergraduate group supervisions
Demonstrator in undergraduate bio-informatics practical sessions
Lead small group supervisions on data handling exercises and journal club research projects for 2nd and 3rd year undergraduate students

2007- **Supervisor for final year undergraduate research projects**
Introduced project background, explained theory behind experimental approaches, demonstrated laboratory techniques, provided constructive feedback on project reports

Collaborations

2016- **Dr Jared Schrader** (Wayne State University – Detroit USA)
Recently established collaboration to study the RNA degradosome in Caulobacter crescentus

2014- **Prof. Rob Lavigne** (KU Leuven, Belgium)
Collaboration to study the structure and function of the phiKZ phage encoded inhibitor of the bacterial RNA degradosome

2012- **Prof Marilis Marques** (University of Sao Paulo, Brazil)
Ongoing collaboration to study role of RNA helicases in Caulobacter crescentus
Joint FAPESP funded travel grant applied for to fund collaboration (2014)

2012- **Prof Hagan Bayley** (University of Oxford, UK)
Collaboration established to examine possibility of using Polynucleotide phosphorylase to aid nano-pore sequencing of RNA
Co-authored publication

2011- **Prof Urs Jenal** (Biozentrum, Universitat Basel, Switzerland)
Collaboration initiated to study in vivo role of RNA degradosome in Caulobacter crescentus
Co-authored publication

Peer Review Experience

I have co-reviewed research papers and reviews submitted to:
Nature, Molecular Cell, Cell Reports, Structure, PLOS Genetics, Biochemistry, Journal of Biological Chemistry, BBA gene regulatory mechanisms, FEBS Journal, Molecular Microbiology, RNA journal, Nucleic Acids Research, Journal of Molecular Microbiology and Biotechnology, Acta Crystallographica section F

Organisational and Administrative Experience

2014 Deputy X-ray crystallography facility manager

2008- Assisted with lab consumables ordering/purchasing

Personal Development

2013- Attended workshops on effective presentation skills as part of Post-doctoral teaching fellows scheme (University of Cambridge)

2013 Workshop for supervising undergraduates (Personnel Dept, University of Cambridge)

2009 British Council organised workshop "Establishing a successful international research career" (Lisbon, Portugal)

Invited Presentations and Conference Attendance

2016 81st Harden Conference: RNA and Disease (Winchester, UK). Talk presented "A novel mechanism for bacteriophage encoded inhibition of the RNA degradosome assembly"

2014 EMBO Workshop: "Stalked alpha-proteobacteria and their relatives" (Marburg, Germany). Talk presented "Molecular Recognition through micro-domains in the *Caulobacter crescentus* RNA degradosome"

2013 **"**Regulating with RNA in Bacteria" (Wurzburg, Germany). Poster presented "Characterisation of the RNA degradosome in *Caulobacter crescentus*."

2012 FASEB meeting "Post-transcriptional control of gene expression: mechanisms of mRNA decay" (Steamboat Springs, Colorado, USA). Talk presented "The RNA degradosome of *Caulobacter crescentus* and its dynamic association with 3'-5' exoribonucleases"

2011 Diamond synchrotron user meeting (Didcot UK). Invited speaker "Improved diffraction of *C. crescentus* PNPase crystals by dehydration with HC1."

2011 Mol Micro meeting Wurzburg (Wurzburg, Germany). Poster presented "The crystal structure of *Caulobacter crescentus* polycnucleotide phosphorylase reveals a potential mechanism for RNA substrate chanelling"

2009 3rd ASM conference on Prokaryotic development (Cambridge, Massachusetts, USA). Poster presented "A multi-enyzme RNA degradative machine from *Caulobacter crescentus.*

2008 FASEB meeting "Post-transcriptional control of gene expression: mechanisms of mRNA decay" (Lucca, Italy). Talk presented **"**Identification of an RNA degradosome complex in *Caulobacter crescentus* that is regulated in a cell cycle dependent manner"

Original Research Publications

2017 Tichy EM**, Hardwick SW**, Luisi BF, Salmond GPC. 1.8 Å resolution crystal structure of the carbapenem intrinsic resistance protein CarF. *Acta Cryst D*. **73**, 549-556

2017 Aguirre AA, Vicente AM, **Hardwick SW**, Alvelos DM, Mazzon RR, Luisi BF, Marques MV. Association of the Cold Shock DEAD-Box RNA Helicase RhlE to the RNA Degradosome in Caulobacter crescentus. *J. Bacteriol*. **199**, e00135-17

2017 Gonzalez GM, **Hardwick SW**, Maslen SL, Skehel JM, Holmqvist E, Vogel J, Bateman A, Luisi BF, Broadhurst RW. Structure of the Escherichia coli ProQ RNA-binding protein. *RNA* **23**, 696-711

2016 Van den Bossche A, **Hardwick SW**, Ceyssens PJ, Hendrix H, Voet M, Dendooven T, Bandyra KJ, De Maeyer M, Aertsen A, Noben JP, Luisi BF, Lavigne R. Structural elucidation of a novel mechanism for the bacteriophage-based inhibition of the RNA degradosome. *Elife* **5**, e16413

2014 Voss J, Luisi BF, **Hardwick SW**. Molecular recognition through microdomains in the Caulobacter crescentus RNA degradosome. *Nucleic Acids Res.* **42**, 13294-305

2013 Ayub M, **Hardwick SW**, Luisi BF, Bayley H. Nanopore-based identification of individual nucleotides for direct RNA sequencing. *Nano Lett*. **13**, 6144-50

2013 Pietras Z, **Hardwick SW**, Swiezewski S, Luisi BF. Potential regulatory interactions of Escherichia coli RraA protein with DEAD-box helicases. *J Biol Chem*. **288**, 31919-29

2012 **Hardwick SW**, Gubbey T, Hug I, Jenal U, Luisi BF. Crystal structure of *Caulobacter crescentus* polynucleotide phosphorylase reveals a mechanism of RNA substrate channeling and RNA degradosome assembly. *Open Biology* 2012 2:120028

2011 **Hardwick SW**, Chan VS, Broadhurst RW, Luisi BF. An RNA degradosome assembly in *Caulobacter crescentus*. *Nucleic Acids Res*. **39**, 1449-59

2009 Pané-Farré J, Jonas B, **Hardwick SW**, Gronau K, Lewis RJ, Hecker M, Engelmann S. Role of RsbU in controlling SigB activity in Staphylococcus aureus following alkaline stress. *J Bacteriol.* **191**, 2561-73

2007 **Hardwick SW**, Pané-Farré J, Delumeau O, Marles-Wright J, Murray JW, Hecker M, Lewis RJ. Structural and functional characterization of partner switching regulating the environmental stress response in Bacillus subtilis. *J Biol Chem*. **282**, 11562-72

2004 Delumeau O, Dutta S, Brigulla M, Kuhnke G, **Hardwick SW**, Völker U, Yudkin MD, Lewis RJ. Functional and structural characterization of RsbU, a stress signaling protein phosphatase 2C. *J Biol Chem*. **279**, 40927-37

Reviews/Points of View

2017 Dendooven T, Van den Bossche A, Hendrix H, Ceyssens PJ, Voet M, Bandyra KJ, De Maeyer M, Aertsen A, Noben JP, **Hardwick SW**, Luisi BF, Lavigne R. Viral interference of the bacterial RNA metabolism machinery. RNA Biol. 14, 6-10

2013 **Hardwick SW**, Luisi BF. Rarely at rest: RNA helicases and their busy contributions to RNA degradation, regulation and quality control. *RNA Biol*. **10**, 56-70

Academic Referees

Professor Ben Luisi, Department of Biochemistry, University of Cambridge, 80 Tennis Court Road, CB2 1GA, UK. 01223-766019, bfl20@cam.ac.uk

Professor Rick Lewis, Institute of Cell and Molecular Biosciences, Newcastle University, UK. 01912085482, rick.lewis@ncl.ac.uk