1. Cryo-Electron Microscopy Equipment grant application

Reference number	206180/Z/17/Z
Applicant name	Dr David Bhella
Title of application	The Scottish Macromolecular Imaging Centre (SMIC)
Total amount requested	£4,000,000.00

2. Application summary

Type of award Please select the type of award you are applying for
Cryo-electron microscopy

Application title
The Scottish Macromolecular Imaging Centre (SMIC)

Proposed duration of funding (months)
60

Proposed start date	08/01/2018

Name of administering organisation
University of Glasgow

Lead applicant's address at administering organisation	
Department/Division	MRC - University of Glasgow Centre for Virus Research
Organisation	University of Glasgow
Street	464 Bearsden Road
City/Town	Glasgow
Postcode/Zipcode	G61 1QH
Country	United Kingdom

Summary of the Equipment, Biomedical Resource or Technology Development for which support is sought.
A 300 keV cryo-electron microscope with 12-slot autoloader, energy-filter, electron counting direct-detector camera, hole-free phase-plates, automated tomography and single-particle data collection

plus five years maintenance contract.

Science stream Indicate the relevant Science stream for the proposed research.
Infection and Immuno-biology

3. Lead applicant

Lead applicant details	
Full Name	Dr David Bhella
Department	MRC - University of Glasgow Centre for Virus Research
Division	College of Medical, Veterinary and Life Sciences
Organisation	University of Glasgow
Address Line 1	MRC - University of Glasgow Centre for Virus Research
City/Town	GLASGOW
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Country	United Kingdom
Telephone No.	014 1330 3685
Email Address	david.bhella@glasgow.ac.uk

ORCID iD	
ORCID iD	0000-0003-2096-8310

Career history (current/most recent first)			
From	**To**	**Position**	**Organisation**
11/2011	09/2036	Programme Leader	University of Glasgow
01/2006	11/2011	Programme Leader Track Scientist	MRC Virology Unit
08/2002	08/2006	Investigator Scientist	MRC Virology Unit
08/1998	08/2002	Research Associate	MRC Virology Unit
10/1991	11/1993	Medical Laboratory Scientific Officer	London Hospital Medical College

Education/training				
From	**To**	**Qualification**	**Subject**	**Organisation**
11/1993	06/1998	Doctor of Philosophy (PhD;DPhil)	Crystallography	Birkbeck University of London
09/1988	06/1991	Bachelor of Science (BSc)	Applied Biology	Cardiff University

Source(s) of personal salary support
Medical Research Council University Unit Core Funding

Clinical status Do you have a medical/veterinary degree?	No

Career breaks Have you had any career breaks or periods of part-time work, for example parental or long-term sick leave?	No

Do you wish to undertake this award part time?	No

Details of any relevant previous experience of managing a resource, a technology or equipment.

I have worked in the field of macromolecular structure determination by cryogenic electron microscopy (CryoEM) for 23 years. I undertook my PhD with Professor Helen Saibil FRS researching the structure of retrotransposon virus-like particles. Following completion of my PhD I moved to the MRC Virology Unit where I was tasked with establishing a CryoEM capability and a programme of structural biology research. In the intervening years I have built an effective cryoEM resource and productive programme of research into virus structure, entry and morphogenesis.

Initial cryoEM research was conducted using an entry-level 120 keV TEM, I specified and sourced appropriate digitisation and computational resources to enable a 3D EM research programme. In 2005, following successful publication of intermediate resolution structures (12-14 angstroms) I led a successful application to MRC for a tomography capable 200 keV FEG cryomicroscope, leading to the installation of our current JEOL 2200 instrument.

Throughout my career in addition to leading a productive scientific programme and having oversight of the CVR cryoEM facility, I have had a hands-on role in the management of our imaging equipment; training users and ensuring operation at the highest standards. I have also maintained our computational resources, identifying appropriate hardware, evaluating image processing software and devising image processing pipelines.

I manage the CVR virus structure group's research infrastructure:

JEOL 2200 CryoEM fitted with Direct Electron DE20 DDD camera and a GATAN Ultrascan 4000 CCD.
JEOL 1200 TEM with GATAN Orius CCD camera.

Preparation equipment:
FEI vitrobot
Leica EMPACT high-pressure freezer
Leica AFS freeze substitution system
Leica UC6 cryo-ultramicrotome
Linkam correlative epifluorescence cryomicroscope.

Computational resources
496 core linux compute cluster
>10 workstations.

These resources have been made available to the Scottish structural biology community to support the wider adoption of this technology. Furthermore I delivered training courses to post-graduate students and post-doctoral researchers from across Scotland. This has led to the foundation of a

network of researchers that is the basis of this consortium application.

I teach on the Royal Microscopical Society cryoEM course.

Former PhD students Kirsty MacLellan and Rebecca Pink-Lauder now run high-quality electron microscopy facilities at NIBSC and Rothamsted respectively.

Career contributions
What are your most important research-related contributions to date? These may include contributions to health policy or practice, or to technology or product discovery and development.

Novel methods for cryoEM imaging of enveloped viruses. We have developed methods for imaging fragile enveloped viruses by cryoEM. By propagating the virus directly on the support film we avoid damage caused by purification protocols. Cryotomography of influenza A virus filaments showed that most long-filaments do not contain genomes, raising questions about the role of the filamentous phenotype *in-vivo* (Vijayakrishnan *et al.* 2013. 20-citations).

Methods for in-situ structure analysis. We are developing methods for *in situ* structure analysis by cryotomography of the frozen-hydrated virus-infected cell. We recently demonstrated that sub-tomogram averaging may be performed on data collected from refrozen Tokayasu sections (in preparation) and have developed a prototype shuttle for performing FIB on cells propagated on the EM grid.

Structure analysis of the Respiratory Syncytial Virus nucleocapsid - The RSV nucleocapsid is a helical assembly comprising the viral genome and the nucleocapsid-protein N, and is the template for viral RNA synthesis. We demonstrated that recombinant expression of RSV N led to formation of N-RNA rings (Bhella *et al.* 2002. 94-citations). In collaboration with Jean-Francois Eleuoet and Felix Rey, these were crystallised. Our EM map (MacLellan *et al.* 2007. 22-citations) allowed phasing of X-ray data to produce an atomic structure. Tomography informed the calculation of a helical model of the assembly.

Structural characterisation of a virus-host interaction that affects adenovirus tropism - In collaboration with Professor Andrew Baker (University of Glasgow) we identified a specific interaction between the adenovirus major capsid protein (hexon) and a protein of the coagulation cascade factor X (FX). This interaction had been the subject of extensive study by the adenovirus gene therapy research community as it had been shown to redirect vector to the liver. Our analysis by cryoEM unambiguously identified hexon as the target of FX (Waddington *et al.* 2008 - 461-citations). Although the density was confounding (owing to the 1:3 stochiometry of FX:Hexon), we succesfully modelled the interaction (supplemental materials) and produced a list of putative contact residues in hexon that was later tested by site directed mutagenesis yielding virus that did not bind FX or transduce hepatocytes (Alba *et al.* 2009 - 121-citations).

Peer-reviewed publications and other research outputs
List up to 20 of your most significant peer-reviewed publications or other scholarly contributions and other research outputs, e.g. patents. Please ensure that at least five of these are from the last five years. For 10 of these outputs, you may also provide a statement describing their significance (up to 50 words per output).

For original research publications indicate those arising from Trust-funded grants in **bold**, and provide the PMCID reference for each of these. Please refer to guidance notes.

*Please give citation in full, including title of paper and all authors**
*(*All authors, unless more than 10, in which case please use 'et al', ensuring that your position as author remains clear.)*

Cryotomography of budding influenza A virus filaments Highlighted by faculty of 1000 (http://f1000.com/prime/718018378). We demonstrated that long filamentous particles do not, in general, contain structures consistent with the presence of viral genomes. We proposed several hypotheses for the role of these particles. The filamentous trait has been shown to be critical for virus transmission in-vivo.

Vijayakrishnan, S., C. Loney, D. Jackson, W. Suphamungmee, F. J. Rixon and D. Bhella 2013. Cryotomography of budding Influenza A Virus reveals filaments with diverse morphologies that mostly do not bear a genome at their distal end. PLoS Pathogens 9(6): e1003413.

Structure and packing of paramyxovirus and pneumovirus nucleocapsids. Our electron microscopy studies of nucleocapsid-like structures produced by recombinant expression of N have led to the calculation of an atomic-resolution model of the helical RSV nucleocapsid, showing how the genome is encapsidated and allowing the formulation of testable hypotheses on how the polymerase may interact with the viral genome.

Bakker, S.E., S. Duquerroy, C. Loney, E. Conner, F. A. Rey and D. Bhella 2013. The Respiratory Syncytial Virus nucleoprotein-RNA complex forms a left-handed helical nucleocapsid. Journal of General Virology 94: 1734–1738.

Tawar, R. G., S. Duquerroy, C. Vonrhein, P. F. Varela, L. Damier-Piolle, N. Castagne, K. MacLellan, H. Bedouelle, G. Bricogne, D. Bhella, J. F. Eleouet, and F. A. Rey. 2009. Crystal Structure of a Nucleocapsid-Like Nucleoprotein-RNA Complex of Respiratory Syncytial Virus. Science 326: 1279-1283.

Loney, C., G. Mottet-Osman, L. Roux, and D. Bhella. 2009. Paramyxovirus Ultrastructure and Genome Packaging: Cryo-Electron Tomography of Sendai Virus. Journal of Virology 83: 8191-8197.

MacLellan, K., C. Loney, R. P. Yeo, and D. Bhella. 2007. The 24-angstrom structure of respiratory syncytial virus nucleocapsid protein-RNA decameric rings. Journal of Virology 81: 9519-9524.

Bhella, D., A. Ralph, and R. P. Yeo. 2004. Conformational flexibility in recombinant measles virus nucleocapsids visualised by cryo-negative stain electron microscopy and real-space helical reconstruction. Journal of Molecular Biology 340: 319-331.

Bhella, D., A. Ralph, L. B. Murphy, and R. P. Yeo. 2002. Significant differences in nucleocapsid morphology within the Paramyxoviridae. Journal of General Virology 83: 1831-1839.

Structure analysis of calcivirus attachment and entry Virus attachment is the first stage of the infectious process. We determined the structure of feline calicivirus decorated with a soluble fragment of its cellular receptor - Junctional adhesion molecule A (fJAM-A). We have demonstrated that receptor binding induces conformational changes in the capsid that may prime virus for uncoating in the endosome.

Bhella, D and I.G. Goodfellow, 2011. The cryo-EM structure of Feline Calicivirus bound to Junctional Adhesion Molecule A at 9 Angstroms resolution reveals receptor induced flexibility and

two distinct conformational changes in the capsid protein VP1. Journal of Virology 85: 11381-11390. **WT funding to IGG PMCID: PMC3194967**

Bhella, D., D. Gatherer, Y. Chaudhry, R. Pink, and I. G. Goodfellow. 2008. Structural insights into calicivirus attachment and uncoating. Journal of Virology 82: 8051-8058. **WT funding to IGG PMCID: PMC2519574**

Structural characterisation of a tropism defining interaction between adenovirus and coagulation factor X Adenoviruses have been widely used as experimental gene transfer agents, however it has been shown that following intravascular delivery, virus is targetted to the liver. This is a consequence of an interaction with blood coagulation factor X (FX). We demonstrated that FX binds directly to the major capsid protein (hexon), using cryoEM to solve the structure of FX decorated virus. We docked high-resolution coordinates to build a quasi-atomic resolution model of the interaction - defining putative contact residues. Site directed mutagenesis of the binding site led to the production of mutant virus for which FX binding was ablated.

Alba, R., A. C. Bradshaw, A. L. Parker, D. Bhella, S. N. Waddington, S. A. Nicklin, N. van Rooijen, J. Custers, J. Goudsmit, D. H. Barouch, J. H. McVey, and A. H. Baker. 2009. Identification of coagulation factor (F)X binding sites on the adenovirus serotype 5 hexon: effect of mutagenesis on FX interactions and gene transfer. Blood 114: 965-971.

Waddington, S. N., J. H. McVey, D. Bhella, A. L. Parker, K. Barker, H. Atoda, R. Pink, S. M. K. Buckley, J. A. Greig, L. Denby, J. Custers, T. Morita, I. M. B. Francischetti, R. Q. Monteiro, D. H. Barouch, N. van Rooijen, C. Napoli, M. J. E. Hlavenga, S. A. Nicklin, and A. H. Baker. 2008. Adenovirus serotype 5 hexon mediates liver gene transfer. Cell 132: 397-409.

Cryomicroscopy of echovirus-receptor interactions Echoviruses (EV) attach to the host-cell surface by binding the complement control protein decay-accelerating factor (CD55). CD55 comprises four 'short consensus repeat' (SCRs) domains. We used cryo-negative stain and icosahedral reconstruction to determine the structure of EV12 bound to soluble fragments of CD55 comprising SCR 3-4 and SCR1-4, identifying contact residues residing primarily in SCR3. Our analysis corrected an erroneous model previously deposited in the protein data-bank by the laboratory of Professor Michael Rossmann.

Pettigrew, D. M., D. T. Williams, D. Kerrigan, D. J. Evans, S. M. Lea, and D. Bhella. 2006. Structural and functional insights into the interaction of echoviruses and decay-accelerating factor. Journal of Biological Chemistry 281: 5169-5177.

Bhella, D., I. G. Goodfellow, P. Roversi, D. Pettigrew, Y. Chaudhry, D. J. Evans, and S. M. Lea. 2004. The structure of echovirus type 12 bound to a two-domain fragment of its cellular attachment protein decay-accelerating factor (CD 55). Journal of Biological Chemistry 279: 8325-8332.

Other relevant publications

Conley, M. J., E. Emmott, R. Orton, D. Taylor, D. G. Carneiro, K. Murata, I. G. Goodfellow, G. Hansman, D Bhella 2016. Vesivirus 2117 capsids more closely resemble sapovirus and lagovirus particles than other known vesivirus structures. Journal of General Virology (in press) **WT funding**

to IGG

McGonigle R., W. B. Yap, S. T. Ong, D. Gatherer, S. E. Bakker, W. S. Tan, D. Bhella 2015. An N-terminal extension to the hepatitis B virus core protein forms a poorly ordered trimeric spike in assembled virus-like particles. Journal of Structural Biology 189: 73-80

Fan W.H., A.P. Roberts, M. McElwee, D. Bhella, F.J. Rixon, R. Lauder 2015. The Large Tegument Protein pUL36 is Essential for Formation of the Capsid Vertex Specific Component at the Capsid-Tegument Interface of HSV-1. Journal of Virology 89: 1502-1511

Schmid, M.F., C.W. Hecksel, R.H. Rochat, D. Bhella, W. Chiu and F.J. Rixon 2012. A Tail-like Assembly at the Portal Vertex in Intact Herpes Simplex Type-1 Virions. PLoS Pathogens 8(10): e1002961.

Parsons, J. B., S. Frank, D. Bhella, M. Z. Liang, M. B. Prentice, D. P. Mulvihill, and M. J. Warren. 2010. Synthesis of Empty Bacterial Microcompartments, Directed Organelle Protein Incorporation, and Evidence of Filament-Associated Organelle Movement. Molecular Cell 38: 305-315.

Bhella, D., F. J. Rixon, and D. J. Dargan. 2000. Cryomicroscopy of human cytomegalovirus virions reveals more densely packed genomic DNA than in herpes simplex virus type 1. Journal of Molecular Biology 295: 155-161.

Al-Khayat, H. A., D. Bhella, J. M. Kenney, J. F. Roth, A. J. Kingsman, E. Martin-Rendon, and H. R. Saibil. 1999. Yeast Ty retrotransposons assemble into virus-like particles whose T-numbers depend on the C-terminal length of the capsid protein. Journal of Molecular Biology 292: 65-73.

Total number of peer-reviewed publications which you have authored/co-authored. Please exclude abstracts and literature reviews.	34

Are you a named author on any Wellcome Trust funded original peer-reviewed research papers, published from October 2009 onwards?	Yes

Are all your Wellcome Trust funded original peer-reviewed research papers, published from October 2009 onwards, compliant with our open access policy?	Yes

Current research funding (including Wellcome Trust grants)
Please list all active grants only (starting with the most recently awarded). State the name of the awarding body, name(s) of grantholder(s), title of project, amounts awarded, your role in the project, and start and end dates of support. Indicate the number of hours per week that are spent on each project.

Medical Research Council Zika: Characterisation of Zika virus neutralisation and virion structure by cryogenic electron microscopy and 3D reconstruction ZK/16-012 (7/2016 – 11/2017 Value £133,141)

Medical Research Coucil Core funding to MRC - University of Glasgow Centre for Virus Research - Programme 8 - Virus Structure MC_UU_12014/7 (4/2016 - 4/2021 Value £1,573,546)

National Institutes of Health (USA) Initiation and regulation of RSV mRNA transcription and genome replication (co-investigator) 1R01AI113321 (Dates 7/8/2014-31/7/2018 Value $190,789)

Medical Research Council A structure analysis of the intact virion and replicative complexes of human respiratory syncytial virus MR/M000451/1 (Dates: 30/11/2014-29/11/2017 Value: £751,764)

Please describe how the currently active grants listed above relate to this application
All of the above listed grants involve the structural characterisation of important human pathogens by cryogenic electron microscopy. In particular MR/M000451/1 aims to determine the structure of the RSV RNA-dependent RNA polymerase - a critical component of pneumovirus replicative assemblies. We have previously determined the structure of the polymerase template; the RSV nucleocapsid. MR/M000451/1 and MC_UU_12014/7 will use phase-plate tomography to determine the structure of filamentous virions formed by RSV and Influenza A as well as exploring methods for *in situ* structure determination using both focussed ion beam milling and ultra-microtomy techiques to investigate the cell interior under cryogenic imaging conditions. 1R01AI113321 funds a PhD studentship - the aim of this project is to perform cryo-tomography of RSV replicative complexes *in situ.* This will also exploit phase plates to achieve high-contrast tomograms. Finally we are exploring the structure of Zika virus virions and virus-like particles decorated with neutralising antibodies with the aim to inform the development of safe and effective vaccines - a collaboration with Drs Alain Kohl and Arvind Patel of the CVR. All projects would benefit immensely from high-performance automated cryoEM or cryotomography with direct-detectors and phase plates.

4. Applicants

1

Applicant	
Full Name	Dr Laura Spagnolo
Department	
Division	
Organisation	University of Glasgow, College of Medical, Veterinary and Life Sciences
Address Line 1	409, Bower Building, University Avenue
City/Town	Glasgow
Postcode	G12 8QQ
Country	United Kingdom
Telephone No.	(0)141 3305133
Email Address	laura.spagnolo@glasgow.ac.uk

Career history (current/most recent first)			
From	**To**	**Position**	**Organisation**
02/2016	12/2030	Reader	University of Glasgow, College of Medical, Veterinary and Life Sciences
08/2014	01/2016	Senior Lecturer	University of Edinburgh
01/2009	08/2014	Lecturer	University of Edinburgh

Education/training

From	To	Qualification	Subject	Organisation
10/1998	02/2002	Doctor of Philosophy (PhD;DPhil)	Pharmaceutical sciences	Universita' di Padova, Italy
10/1992	04/1998	Master of Science (MSc)	Pharmacy	Universita' di Trieste, Italy

Source(s) of personal salary support

University of Glasgow, College of Medical, Veterinary and Life Sciences

Details of any relevant previous experience of managing a resource, a technology or equipment.

In 2007-2008, I was part of the steering committee that oversaw the establishment of the structural electron microscopy initiative at the University of Edinburgh.

I was a co-applicant on both successful Wellcome Trust-funded equipment grant applications that funded the purchase of electron microscopes at the University of Edinburgh:

2009-2014 Establishing high-resolution electron cryo-microscopy in Edinburgh 087658/Z/08/Z;

2015-2020 Advancing our capabilities for visualization of cellular structures and isolated complexes by electron microscopy, 104915/Z/14/Z.

Between 2009 and 2015, I was part of the cryo-electron microscopy facility management group (with Boettcher, Oparka and Kelly).

I am currently overseeing the purchase and installation of an electron microscope for screening purposes at the Gilmorehill campus, University of Glasgow.

Career contributions
What are your most important research-related contributions to date? These may include contributions to health policy or practice, or to technology or product discovery and development.

My electron microscopy studies allowed to elucidate important molecular mechanisms in DNA repair by Non-Homologous End-Joining. In particular, I showed how broken DNA ends are kept together by a large assembly formed by two copies of the assembly formed by DNA-PKcs/Ku70/Ku80 proteins, suggesting how this DNA-threading machine could accommodate further downstream partners to process and ligate the DNA ends.

Electron microscopy coupled to single particle analysis studies also allowed me to show how Type III CRISPR systems share some common backbone features with Type I systems, even though their mechanisms differ quite substantially. Furthermore, I demonstrated how two Sulfolobal Type III systems, CSM and CMR, share an overall architecture (head, tail, backbone and belly), however they are very different in length. This hints for a mutual influence between the size and nature of target nucleic acids and the assembly of the related CRISPR complex.

Although still preliminary, my current electron microscopy work on Staufen protein has highlighted the formation of well-ordered granules of Staufen protein on target RNA. These studies are complemented by the study of Staufen and its interaction with known RNA substrates in solution (SAXS and SANS) as well as in a cellular context (super-resolution microscopy).

Peer-reviewed publications and other research outputs
List up to 20 of your most significant peer-reviewed publications or other scholarly contributions and other research outputs, e.g. patents. Please ensure that at least five of these are from the last five years. For 10 of these outputs, you may also provide a statement describing their significance (up to 50 words per output).

*Please give citation in full, including title of paper and all authors**
*(*All authors, unless more than 10, in which case please use 'et al', ensuring that your position as author remains clear.)*

Cannone, G., Xu, L., Beattie, T. Bell, SD., Spagnolo, L., *"The architecture of the 'Okazakisome' reveals the mechanism for recruitment of multiple client proteins on a single PCNA ring".* ***Biochemical Journal*** 2014 Oct 9. DOI 10.1042/BJ20141120. PMID: 25299633.

Rouillon C., Zhou M., Zhang J., Cannone G., Graham S., Robinson CV.*, **Spagnolo L.***, White MF.*, *"Structure of the CRISPR Surveillance Complex CSM reveals key similarities with Cascade".* ***Molecular Cell***, Volume 52, Issue 1, 124-134, 10 October 2013. *Corresponding Authors. DOI 10.1016/j.molcel.2013.08.020. PMCID: PMC3807668. Cited 53 times.

Featured in "Molecular Cell Minireviews"; *Featured in* "Faculty of 1000 Prime";

Webcast http://www.youtube.com/watch?v=W2JsahSrz0&list=PLKzT3b3RHQwhj40pl8Gj_yrn8o5JPOY49&index=1

Froese D.S., Vollmar M., Puranik S., Cannone G., Savitsky P., Krojer T., Pilka E., Lee W.H., Marsden B.D., von Delft F., Allerston C.K., **Spagnolo L.**, Gileadi O., Oppermann U., Yue W.W. *"Structural basis for the catalytic and disease mechanisms of eukaryotic malonyl-CoA decarboxylase".* ***Structure.*** 2013 Jul 2;21(7):1182-92. DOI: 10.1016/j.str.2013.05.001. PMCID: PMC3701320. Cited 3 times.

LeBihan T.*, Rayner J., Roy M., **Spagnolo L.***, *"Photobacterium profundum under pressure: a label-free MS study"*. ***PLoS One.*** 2013 May 31;8(5):e60897. *Corresponding Authors. DOI: 10.1371/journal.pone.0060897. PMCID: PMC3669370. Cited 6 times.

Zhang J., Rouillon C., Kerou M., Reeks J., Brugger K., Graham S., Reimann J., Cannone G., Liu H., Albers SV., Naismith JH., **Spagnolo L.***, White MF*. *"Structure and function of the CMR complex for CRISPR-mediated antiviral immunity*". ***Molecular Cell.*** 2012 Feb 10;45(3):303-13. *Corresponding Authors. Cover. DOI: 10.1016/j.molcel.2011.12.013. PMCID: PMC3381847. Cited 160 times.

Spagnolo L.*, Barbeau J., Curtin NJ., Morris EP., Pearl LH., *"Visualization of a DNA-PK/PARP1 complex"*, ***Nucleic Acids Research,*** 2012 May 1;40(9):4168-77. *Corresponding Author. DOI: 10.1093/nar/gkr1231. PMCID: PMC3351162. Cited 37 times.

Morris EP, Rivera-Calzada A, da Fonseca PC, Llorca O, Pearl LH, **Spagnolo L**.* *"Evidence for a remodelling of DNA-PK upon autophosphorylation from electron microscopy studies".* ***Nucleic Acids Research***, 2011 Mar 30. *Corresponding Author. DOI: 10.1093/nar/gkr146. PMCID: PMC3141256. Cited 12 times.

Rivera-Calzada A, **Spagnolo L**, Pearl LH, Llorca O. *"Structural model of full-length human Ku70-Ku80 heterodimer and its recognition of DNA and DNA-PKcs".* ***EMBO Reports.*** 2007 Jan;8(1):56-62. DOI: 10.1038/sj.embor.7400847. PMCID: PMC1796749. Cited 73 times.

Spagnolo L, Rivera-Calzada A, Pearl LH, Llorca O. *"Three-dimensional structure of the human DNA-PKcs/Ku70/Ku80 complex assembled on DNA and its implications for DNA DSB repair".* ***Molecular Cell.*** 2006 May 19;22(4):511-9. Cover. DOI: 10.1016/j.molcel.2006.04.013. PMID:

1796749. Cited 160 times.

Featured in “Faculty of 1000”.

Rivera-Calzada A, Maman JD, **Spagnolo L**, Pearl LH, Llorca O. *“Three-dimensional structure and regulation of the DNA-dependent protein kinase catalytic subunit (DNA-PKcs)”.* ***Structure.*** 2005 Feb;13(2):243-55. DOI: 10.1038/sj.embor.7400847. PMID: 1796749. Cited 84 times.

Spagnolo L, Toro I, D'Orazio M, O'Neill P, Pedersen JZ, Carugo O, Rotilio G, Battistoni A, Djinovic-Carugo K. *“Unique features of the sodC-encoded superoxide dismutase from Mycobacterium tuberculosis, a fully functional copper-containing enzyme lacking zinc in the active site”.* ***Journal of Biological Chemistry.*** 2004 Aug 6;279(32):33447-55. DOI: 10.1074/jbc.M404699200. PMID: 15155722. Cited 54 times.

Spagnolo L, Ventura S, Serrano L. *“Folding specificity induced by loop stiffness”.* ***Protein Science.*** 2003 Jul;12(7):1473-82. DOI: 10.1110/ps.0302603. PMCID: PMC2323927. Cited 7 times.

Ventura S, Vega MC, Lacroix E, Angrand I, **Spagnolo L**, Serrano L. *“Conformational strain in the hydrophobic core and its implications for protein folding and design”.* ***Nature Structural Biology.*** 2002 Jun;9(6):485-93. DOI: 10.1038/nsb799. PMID: 12824493. Cited 99 times.

Featured in “Faculty of 1000”.

Review Article

Cannone G, Webber-Birungi M, Spagnolo L. “*Electron microscopy studies of Type III CRISPR machines in Sulfolobus solfataricus”.* ***Biochem Soc Trans.*** 2013 Dec;41(6):1427-30. DOI: 10.1042/BST20130166. PMID: 24256232. Review.

Total number of peer-reviewed publications which you have authored/co-authored. Please exclude abstracts and literature reviews.	13

Current research funding (including Wellcome Trust grants)
Please list all active grants only (starting with the most recently awarded). State the name of the awarding body, name(s) of grantholder(s), title of project, amounts awarded, your role in the project, and start and end dates of support. Indicate the number of hours per week that are spent on each project.

PI on **BBSRC BB/J005673/2** Project grant, 03.2012-04.2017 (currently seeking no-cost extension). 444,633.92 GBP. *Elucidating the molecular architecture of the Archaeal CMR complex, a key player in the unicellular immune response.* 10 hours/week.

Co-I on **MRC MR/M000648/1** Project grant, 11.2014-11.2017 (PI MacNeill, St Andrews). 470,906 GBP. *Determining the macromolecular structure and cellular function of an alternative MCM complex.* 2h/week.

Please describe how the currently active grants listed above relate to this application

Both the BBSRC and MRC grants aim at achieving high-resolution structural information on large macromolecular machines using cryo-electron microscopy coupled to image processing. My PhD student Silvia Visentin (funded on an ISIS, STFC studentship) also works on a project that

combines the use of cryo-EM and small angle scattering.

2

Applicant	
Full Name	Dr Danny Huang
Department	Beatson Institute
Division	
Organisation	University of Glasgow
Address Line 1	Garscube Estate, Switchback Road
City/Town	Glasgow
Postcode	G61 1BD
Country	United Kingdom
Telephone No.	0141 3308145
Email Address	d.huang@beatson.gla.ac.uk

Career history (current/most recent first)			
From	**To**	**Position**	**Organisation**
03/2015	03/2020	Senior Group Leader	Cancer Research UK Beatson Institute
03/2009	02/2015	Junior Group Leader	The Beatson Institute for Cancer Research
02/2007	02/2009	Staff scientist	St Jude Children’s Research Hospital, USA
08/2002	02/2007	Posdoctoral fellow	St Jude Children’s Research Hospital, USA

Education/training				
From	**To**	**Qualification**	**Subject**	**Organisation**
02/1997	11/2001	Doctor of Philosophy (PhD;DPhil)	Biochemistry, enzymology	University of Sydney
02/1993	12/1996	Bachelor of Science (BSc)	Biochemistry Honours	University of Sydney

Source(s) of personal salary support
Cancer Research UK core funding and European Research Council

Details of any relevant previous experience of managing a resource, a technology or equipment.
I am currently managing the crystallisation facility and X-ray generator at the Cancer Research UK

Beatson Institute.

Career contributions
What are your most important research-related contributions to date? These may include contributions to health policy or practice, or to technology or product discovery and development.

My research focuses on understanding how a small protein modifier, ubiquitin, is activated and conjugated to the substrate via the E1-E2-E3 enzyme cascade. During my postdoc trainng, I have determined series of crystal structures of E1-E2 complexes (Huang et al., 2004, NSMB; Huang et al., 2005, Mol. Cell; Huang et al., 2007, Nature; Huang et al., 2009, Mol. Cell). Collectively, these works revealed how E1 recruits E2 to transfer ubiquitin and how E1-E2 selectively is achieved.

In 2009, I started my group at the Cancer Research UK Beatson Institute. We have determined several structures of RING-type E3s in complexes with various binding partners. We determined the first structure of a RING E3 bound E2 conjugated with ubiquitin (E2~ubiquitin) revealing the mechanism of E2~ubiquitin activation by a RING E3 (Dou et al., 2012a, NSMB). Moreover, we showed non-covalent ubiquitin binding to E2's backside further activates the activity of RING E3 (Buetow et al., 2015, Mol. Cell). We have also shown how post-translational modification of RING E3 by phosphorylation could regulate the ligase activity. We determined series of CBL structure in both native and tyrosine phosphorylated state and showed that tyrosine phosphorylation abolishes autoinhibition and induces dramatic conformational changes to stimulate ligase activity by over 1,000-fold (Dou et al., 2012b, NSMB; Dou et al., 2013, NSMB).

Although my work has predominantly involved X-ray crystallography, I am keen to exploit the emerging capability of cryoEM for high-resolution structure analysis.

Peer-reviewed publications and other research outputs
List up to 20 of your most significant peer-reviewed publications or other scholarly contributions and other research outputs, e.g. patents. Please ensure that at least five of these are from the last five years. For 10 of these outputs, you may also provide a statement describing their significance (up to 50 words per output).

*Please give citation in full, including title of paper and all authors**
*(*All authors, unless more than 10, in which case please use 'et al', ensuring that your position as author remains clear.)*

Peer-reviewed articles

1. Buetow, L., Tria, G., Ahmed, S.F., Hock, A., Dou, H., Sibbet, G.J., Svergun, D.I. and **Huang, D.T.** (2016) Casitas B-lineage lymphoma linker helix mutations found in myeloproliferative neoplasms affect conformation. ***BMC biology***, 14(1): 76.

2. Marcianò, G. and **Huang, D.T.** (2016) Structure of the human histone chaperone FACT Spt16 N-terminal domain. ***Acta Crystallogr. F Struct. Biol. Commun.*** 72, 121-8.

3. Buetow, L., Gabrielsen, M., Anthony, N.G., Dou, H., Patel, A., Aitkenhead, H., Sibbet, G.J., Smith, B.O., and **Huang, D.T.** (2015) Activation of a primed RING E3-E2-ubiquiitn complex by non-covalent ubiquitin. ***Molecular Cell*** 58, 297-310.

This work reveals how ubiquitin could stabilize a RING E3-E2~ubiquitin complex to further activate the ligase activity.

4. Dou, H., Buetow, L., Sibbet, G.J., Cameron, K. and **Huang, D.T.** (2013) Essentiality of a non-RING element in priming donor ubiquitin for catalysis by a monomeric E3. ***Nature Structural and Molecular Biology*** 20, 982-6.

This work reveals how a phosphotyrosine could directly participate in stabilization of donor ubiquitin to promote catalysis.

5. Dou, H., Buetow, L., Sibbet, G.J., Cameron, K. and **Huang, D.T.** (2012) BIRC7–E2 ubiquitin conjugate structure reveals the mechanism of ubiquitin transfer by a RING dimer. ***Nature Structural and Molecular Biology*** 19, 876-83. (News and Views, *Nature* 489, 43-44).

This work provides the first structural insight into how a RING E3 could activate E2~ubiquitin conjugate for catalysis.

6. Dou, H., Buetow, L., Hock, A., Sibbet, G.J., Vousden, K.H. and **Huang, D.T.** (2012) Structural basis for autoinhibition and phosphorylation-dependent activation of c-Cbl. ***Nature Structural and Molecular Biology*** 19, 184-92. (News and Views, *Nature Structural and Molecular Biology* 19, 131-3).

This work shows that tyrosine phosphorylation activates CBL ubiquitin ligase activity by inducing a dramatic conformational change that abolishes autoinhibition and facilitates juxtaposition of E2~ubiquitin conjugate and substrate.

7. Wang, J, Taherbhoy, A.M., Hunt, H.W., Sevedin, S.N., Miller, D.M., **Huang, D.T.** and Schulman, B.A. (2010) Crystal Structure of UBA2-Ubc9: Insights into E1-E2 Interactions in Sumo Pathways. ***PLoS One***, 5(12): e15805.

8. **Huang, D.T.,** Ayrault, O. Hunt, H.W., Taherbhoy, A.M., Duda, D.M., Scott, D.C., Borg, L.A., Neale, G., Murray, P.J., Roussel, M.F. and Schulman, B.A. (2009) E2-RING expansion of the NEDD8 cascade confers specificity to cullin modification. ***Molecular Cell,*** 33, 483-95.

This work identifies a second NEDD8 E2 and demonstrates that the two NEDD8 E2s displayed distinct specificity for cullin modification.

9. **Huang, D.T.,** Zhuang, M., Ayrault, O. and Schulman, B.A. (2008) Identification of conjugation specificity determinants unmasks vestigial preference for ubiquitin within the NEDD8 E2. ***Nature Structural and Molecular Biology****,* 15, 280-7.

This work shows that NEDD8 E2 evolves from ubiquitin E2 with select amino acid substitutions that alters its specificity for NEDD8 E1.

10. **Huang, D.T.**, Hunt, H.W., Zhuang, M., Ohi, M.D. Holton, J.M. and Schulman, B.A. (2007) Basis for a ubiquitin-like protein thioester switch toggling E1–E2 affinity. ***Nature*** *(Article)* 445, 394-8. (News and Views, *Nature* 445, 375-6).

This work provides the first structural insight into how E1~Ub thioester formation could alter E1 conformation to enhance E2 binding.

11. **Huang, D.T.,** Kaplan, J., Menz, R.I., Katis, V.L., Wake, R.G., Zhao, F., Wolfenden, R. and Christopherson R.I. (2006) Thermodynamic analysis of catalysis by the dihydroorotases from hamster and *Bacillus caldolyticus*, as compared with the uncatalyzed reaction. ***Biochemistry***, 45, 8275-83.

12. Anderson, M.A., Cleland, W.W., **Huang, D.T.**, Chan, C., Shojaei, M. and Christopherson, R.I. (2006) ^{13}C and ^{15}N Isotope effects for conversion of L-dihydroorotate to *N*-carbamyl-L-aspartate using dihydroorotase from hamster and *Bacillus caldolyticus. **Biochemistry***, 45, 7132-39.

13. Eletr, Z.M.[1], **Huang, D.T**.[1], Duda, D.M., Schulman, B.A. and Kuhlman, B. (2005) E2 Conjugating Enzymes Must Disengage from their E1 Activating Enzyme before E3-dependent Ubiquitin and Ubiquitin-like Transfer. ***Nature Structural and Molecular Biology****,* 12, 933-4.
[1]These authors contributed equally to the work.

This work shows that E2 must disengage from E1 before interacting with E3.

14. **Huang, D.T.**, Paydar, A., Zhuang, M., Waddell, M.B., Holton, J.M. and Schulman, B.A. (2005) Structural basis for recruitment of Ubc12 by an E2-binding domain in NEDD8's E1. ***Molecular Cell.*** 17, 341-350. (Preview, *Molecular Cell* 17, 474-5, 2005).

This work provides the first structural insight into E1-E2 interaction.

15. **Huang, D.T.**, Miller, D.W., Mathew, R., Cassell, R., Holton, J.M., Roussel, M.F. and Schulman, B.A. (2004) A unique E1-E2 interaction required for optimal conjugation of the ubiquitin-like protein NEDD8. ***Nature Structural and Molecular Biology***, 11, 927-35. (News and Views, *Nature Structural and Molecular Biology* 11, 908-9).

This work identified a unique extension on NEDD8 E2 that is crucial for NEDD8 E1 interaction.

16. Walden, H, Podgorski, M.S., **Huang, D.T.**, Miller, D.W., Howard, R.J., Minor, D.L. Jr, Holton, J.M. and Schulman, B.A. (2003) The structure of the APPBP1-UBA3-NEDD8-ATP complex reveals the basis for selective ubiquitin-like protein activation by an E1. ***Molecular Cell***. 12, 1427-37.

Review Articles

1. Buetow, L. and **Huang, D.T.** (2016) Structural insights into the catalysis and regulation of E3 ubiquitin ligases. ***Nat. Rev. Mol. Cell. Biol.*** doi: 10.1038/nrm.2016.91.

2. Nakasone, M.A. and **Huang, D.T.** (2016) Ubiquitination accomplished: E1 and E2 enzymes were not necessary. ***Molecular Cell*** 62, 807-9.

3. **Huang, D.T.** and Schulman, B.A. (2006) Breaking up with a kinky SUMO. ***Nature Structural and Molecular Biology***, 13, 1045-47.

4. **Huang, D.T.**, Walden, H, Duda, D.M. and Schulman, B.A. Ubiquitin-like protein activation. (2004) ***Oncogene*** 23, 1958-71.

Total number of peer-reviewed publications which you have authored/co-authored. Please exclude abstracts and literature reviews.	20

Current research funding (including Wellcome Trust grants)
Please list all active grants only (starting with the most recently awarded). State the name of the awarding body, name(s) of grantholder(s), title of project, amounts awarded, your role in the project, and start and end dates of support. Indicate the number of hours per week that are spent on each project.

European Research Council: Structural and mechanistic insights into RING E3-mediated ubiquitination (Consolidator grant number 647849; 05/2016-05/2020; value 2,000,000 Euro). Principal investigator, 20 hours/week

Cancer Research UK core funding: Structural characterisation of ubiquitin ligases (03/2016-03/2020 value £2,000,000) Principal investigator 20 hours/week

Please describe how the currently active grants listed above relate to this application

Both grants listed above involve structural characterisation of various ubiquitin ligases in complex with their binding partners. While X-ray crystallography is the primary method for structural

determination, the advances in CryoEM over the last few years enable us to explore the possibility of structural characterisation by this method via collaboration with Dr David Bhella. Several complexes in the grant listed above are in the range of 300-600 kDa and would benefit immensely from a high performance CryoEM.

3

Applicant	
Full Name	Prof James Naismith
Department	BSRC
Division	
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Career history (current/most recent first)			
From	**To**	**Position**	**Organisation**
01/1995	08/2033	Professor (Reader, Lecturer)	University of St Andrews
01/1993	12/1994	PDRA	UTSW

Education/training				
From	**To**	**Qualification**	**Subject**	**Organisation**
10/1989	11/1992	Doctor of Philosophy (PhD;DPhil)	Structural Biology	University of Manchester
09/1985	06/1989	Bachelor of Science (BSc)	Chemistry	University of Edinburgh

Source(s) of personal salary support
SFC

Details of any relevant previous experience of managing a resource, a technology or equipment.
I managed as the (elected head) the CCP4 (the collaborative computing project that underpins protein crystallographic software development in the UK) for 9 years, renewing the funding twice. As Director of the BSRC at St Andrews I secured the funding from Wellcome and University for an £18M project and managed it to completion, on budget, ahead of schedule and exceeded performance targets. I am currently the elected head of the SFX consortium which is building a dedicated protein crystallography beamline at the European X-FEL. I am also the Head of the UK

Membrane Protein Laboratory which has just secured funding from the Wellcome Trust.

Career contributions
What are your most important research-related contributions to date? These may include contributions to health policy or practice, or to technology or product discovery and development.

I believe my most important contributions have been to advance a structural and chemical understanding of the processes involved in the biosynthesis of bacterial secondary metabolites and LPS component molecules. This work has led onto a spin out company and to high throughput screening to identify new drugs for bacteria. I think my work has encouraged other labs to bring chemistry to bear in the study of enzymes.

Peer-reviewed publications and other research outputs
List up to 20 of your most significant peer-reviewed publications or other scholarly contributions and other research outputs, e.g. patents. Please ensure that at least five of these are from the last five years. For 10 of these outputs, you may also provide a statement describing their significance (up to 50 words per output).

*Please give citation in full, including title of paper and all authors**
*(*All authors, unless more than 10, in which case please use 'et al', ensuring that your position as author remains clear.)*

1 Pliotas **C, Dahl AC, Rasmussen T, Mahendran KR, Smith TK, Marius P, Gault J, Banda T, Rasmussen A, Miller S, Robinson CV, Bayley H, Sansom MS, Booth IR, Naismith JH. The role of lipids in mechanosensation. Nat Struct Mol Biol. 2015 Dec;22(12):991-8. doi: 10.1038/nsmb.3120. Epub 2015 Nov 9. PubMed PMID: 26551077; PubMed Central PMCID: PMC4675090.**

2 Branigan E, Plechanovova´ A, Jaffray EG, Naismith JH, Hay RT. Structural basis for the RING-catalyzed synthesis of K63-linked ubiquitin chains. Nat Struct Mol Biol. 2015 Aug;22(8):597-602. doi: 10.1038/nsmb.3052. Epub 2015 Jul 6. PubMed PMID: 26148049; PubMed Central PMCID: PMC4529489.

3 Koehnke J, Mann G, Bent AF, Ludewig H, Shirran S, Botting C, Lebl T, Houssen WE, Jaspars M, Naismith JH. Structural analysis of leader peptide binding enables leader-free cyanobactin processing. Nat Chem Biol. 2015 Aug;11(8):558-63. doi: 10.1038/nchembio.1841. Epub 2015 Jun 22. PubMed PMID: 26098679; PubMed Central PMCID: PMC4512242.

4 Hagelueken G, Clarke BR, Huang H, Tuukkanen A, Danciu I, Svergun DI, Hussain R, Liu H, Whitfield C, Naismith JH. A coiled-coil domain acts as a molecular ruler to regulate O-antigen chain length in lipopolysaccharide. Nat Struct Mol Biol. 2015 Jan;22(1):50-6. doi: 10.1038/nsmb.2935. Epub 2014 Dec 15. PubMed PMID: 25504321; PubMed Central PMCID: PMC4650267.

5 Bushell SR, Mainprize IL, Wear MA, Lou H, Whitfield C, Naismith JH. Wzi **is an outer membrane lectin that underpins group 1 capsule assembly in Escherichia coli. Structure. 2013 May 7;21(5):844-53. doi: 10.1016/j.str.2013.03.010. Epub 2013 Apr 25. PubMed PMID: 23623732; PubMed Central PMCID: PMC3791409.**

6 Alphey MS, Pirrie L, Torrie LS, Boulkeroua WA, Gardiner M, Sarkar A, Maringer M, Oehlmann W, Brenk **R, Scherman MS, McNeil M, Rejzek M, Field RA, Singh M, Gray D, Westwood NJ, Naismith JH. Allosteric competitive inhibitors of the glucose-1-phosphate thymidylyltransferase (RmlA) from Pseudomonas aeruginosa. ACS Chem Biol. 2013 Feb 15;8(2):387-96. doi: 10.1021/cb300426u. Epub 2012 Nov 28. PubMed PMID: 23138692.**

7 Pliotas **C, Ward R, Branigan E, Rasmussen A, Hagelueken G, Huang H, Black SS, Booth IR, Schiemann O, Naismith JH. Conformational state of the MscS mechanosensitive channel in solution revealed by pulsed electron-electron double resonance (PELDOR) spectroscopy. Proc Natl Acad Sci U S A. 2012 Oct 2;109(40):E2675-82. doi: 10.1073/pnas.1202286109. Epub 2012 Sep 10. PubMed PMID: 23012406; PubMed Central PMCID: PMC3479538.**

8 Plechanovova´ A, Jaffray EG, Tatham MH, Naismith JH, Hay RT. Structure **of a RING E3 ligase and ubiquitin-loaded E2 primed for catalysis. Nature. 2012 Sep 6;489(7414):115-20. doi: 10.1038/nature11376. PubMed PMID: 22842904; PubMed Central PMCID: PMC3442243**

9 Koehnke J, Bent A, Houssen WE, Zollman D, Morawitz F, Shirran S, Vendome J, Nneoyiegbe AF, Trembleau L, Botting CH, Smith MC, Jaspars M, Naismith JH. The mechanism of patellamide macrocyclization revealed by the characterization of the PatG macrocyclase domain. Nat Struct Mol Biol. 2012 Aug;19(8):767-72. doi: 10.1038/nsmb.2340. Epub 2012 Jul 15. PubMed PMID: 22796963; PubMed Central PMCID: PMC3462482.

10 Lou H, Chen M, Black SS, Bushell SR, Ceccarelli M, Mach T, Beis K, Low AS, Bamford VA, Booth IR, Bayley H, Naismith JH. Altered antibiotic transport in OmpC mutants isolated from a series of clinical strains of multi-drug resistant E. coli. PLoS One. 2011;6(10):e25825. doi: 10.1371/journal.pone.0025825. Epub 2011 Oct 28. PubMed PMID: 22053181; PubMed Central PMCID: PMC3203869.

11 Plechanovova´ A, Jaffray EG, McMahon SA, Johnson KA, Navra´tilova´ I, Naismith JH, Hay RT. Mechanism of ubiquitylation by dimeric RING ligase RNF4. Nat Struct Mol Biol. 2011 Aug 21;18(9):1052-9. doi: 10.1038/nsmb.2108. PubMed PMID: 21857666; PubMed Central PMCID: PMC3326525.

12 Oke M, Carter LG, Johnson KA, Liu H, McMahon SA, Yan X, Kerou M, Weikart ND, Kadi N, Sheikh MA, Schmelz S, Dorward M, Zawadzki M, Cozens C, Falconer H, Powers H, Overton IM, van Niekerk CA, Peng X, Patel P, Garrett RA, Prangishvili D, Botting CH, Coote PJ, Dryden DT, Barton GJ, Schwarz-Linek U, Challis GL, Taylor GL, White MF, Naismith JH. The Scottish Structural Proteomics Facility: targets, methods and outputs. J Struct Funct Genomics. 2010 Jun;11(2):167-80. doi: 10.1007/s10969-010-9090-y. Epub 2010 Apr 24. PubMed PMID: 20419351; PubMed Central PMCID: PMC2883930.

13 Schmelz S, Kadi N, McMahon SA, Song L, Oves-Costales D, Oke M, Liu H, Johnson KA, Carter LG, Botting CH, White MF, Challis GL, Naismith JH. AcsD catalyzes enantioselective citrate desymmetrization in siderophore biosynthesis. Nat Chem Biol. 2009 Mar;5(3):174-82. doi: 10.1038/nchembio.145. Epub 2009 Feb 1. PubMed PMID: 19182782; PubMed Central PMCID: PMC2644304.

14 Wang W, Black SS, Edwards MD, Miller S, Morrison EL, Bartlett W, Dong C, Naismith JH, Booth IR. The structure of an open form of an E. coli mechanosensitive channel at 3.45 A resolution. Science. 2008 Aug 29;321(5893):1179-83. doi: 10.1126/science.1159262. PubMed PMID: 18755969; PubMed Central PMCID: PMC3299565.

15 Liu H, Rudolf J, Johnson KA, McMahon SA, Oke M, Carter L, McRobbie AM, Brown SE, Naismith JH, White MF. Structure of the DNA repair helicase XPD. Cell. 2008 May 30;133(5):801-12. doi: 10.1016/j.cell.2008.04.029. PubMed PMID: 18510925; PubMed Central PMCID: PMC3326533.

16 Dong C, Beis K, Nesper J, Brunkan**-Lamontagne AL, Clarke BR, Whitfield C, Naismith JH. Wza the translocon for E. coli capsular polysaccharides defines a new class of membrane protein. Nature. 2006 Nov 9;444(7116):226-9. Epub 2006 Nov 1. PubMed PMID: 17086202; PubMed Central PMCID: PMC3315050**

17 Dong C, Flecks S, Unversucht S, Haupt C, van Pe´e KH, Naismith JH. Tryptophan 7-halogenase

(PrnA) structure suggests a mechanism for regioselective chlorination. Science. 2005 Sep 30;309(5744):2216-9. PubMed PMID: 16195462; PubMed Central PMCID: PMC3315827

18 Dong C, Huang F, Deng H, Schaffrath C, Spencer JB, O'Hagan D, Naismith JH. Crystal structure and mechanism of a bacterial fluorinating enzyme. Nature. 2004 Feb 5;427(6974):561-5. PubMed PMID: 14765200.

19 Sanders DA, Staines AG, McMahon SA, McNeil MR, Whitfield C, Naismith JH. UDP-galactopyranose mutase has a novel structure and mechanism. Nat Struct Biol. 2001 Oct;8(10):858-63. PubMed PMID: 11573090.

20 Giraud MF, Leonard GA, Field RA, Berlind C, Naismith JH. RmlC, the third enzyme of dTDP-L- **rhamnose pathway, is a new class of epimerase. Nat Struct Biol. 2000 May;7(5):398-402. PubMed PMID: 10802738.**

Total number of peer-reviewed publications which you have authored/co-authored. Please exclude abstracts and literature reviews.	200

Current research funding (including Wellcome Trust grants)
Please list all active grants only (starting with the most recently awarded). State the name of the awarding body, name(s) of grantholder(s), title of project, amounts awarded, your role in the project, and start and end dates of support. Indicate the number of hours per week that are spent on each project.

1-7-13	30-6-20	2,085,352	Transport and polymerisation of bacterial polysaccharides: from cytoplasm to the outside world		WT 100209/Z/12/Z
1-9-13	30-8-17	222,700	TRANSLOCATION	EUROPEAN COMMISSION	PITN-GA-2013-607694
1-3-14	28-2-19	1,542,366	New Chemical Biology for Tailoring Novel Therapeutics	EUROPEAN COMMISSION	339367-NCB_TNT

I am the Principal Applicant

Please describe how the currently active grants listed above relate to this application

These grants are in the area of structural biology, the cryo EM requested is a tool for structural biology.

Time spent on research
How many hours per week do you spend on research?

40

How many hours per week will be spent on this project?

3

4

Applicant	
Full Name	Prof Tom Owen-Hughes

Department	The Centre for Gene Regulation and Expression
Division	School of Life Sciences
Organisation	University of Dundee
Address Line 1	School of Life Sciences
City/Town	DUNDEE
Postcode	DD1 5EH
Country	United Kingdom
Telephone No.	013 8238 5796
Email Address	t.a.owenhughes@dundee.ac.uk

Career history (current/most recent first)			
From	**To**	**Position**	**Organisation**
12/2007	01/2033	Professor	University of Dundee
01/2001	11/2007	Reader	University of Dundee
01/1998	12/2000	Senior Lecturer	Univeristy of Dundee
01/1994	12/1997	Postdoctoral Fellow	Pennsylvania State University

Education/training				
From	**To**	**Qualification**	**Subject**	**Organisation**
09/1989	07/1993	Doctor of Philosophy (PhD;DPhil)	Biochemistry	University of Oxford
10/1984	07/1988	Bachelor of Science (BSc)	Molecular Biology	University of Edinburgh

Source(s) of personal salary support
50% Wellcome Trust 50% SHEFC

Details of any relevant previous experience of managing a resource, a technology or equipment.
I established a 200keV cryo feeder facility in Dundee and am responsible for its ongoing management. I am responsible for strategic investment to support microscopy, proteomics and high performance computing using funding from a Wellcome Trust “technology platforms” strategic award. I sit on a management committee to oversea the provision of bioinformatics support across the school of life sciences in Dundee.

Career contributions
What are your most important research-related contributions to date? These may include contributions to health policy or practice, or to technology or product discovery and development.

The central theme for my research has been understanding how ATP-dependent chromatin remodelling enzymes act to reconfigure chromatin so as to allow genes to be regulated appropriately. We developed biochemical and biophysical assays that directly reported on how the enzymes distort DNA (Havas et al., 2000; Lia et al., 2006). We then showed that distortion of DNA is coupled to distinct modes of nucleosome alteration (Whitehouse et al., 1999; Flaus and Owen-Hughes 2003; Stockdale et al., 2006; Ahel et al., 2009). Alignment of the sequences of remodelling ATPases showed how they could be classified into subfamilies that correlated with enzyme activity assessed both by colleagues and ourselves (Flaus et al., 2006).

We crystallised the C-terminal DNA binding domain of Chd1 (Ryan et al., 2011) revealing structurally conserved SANT and SLIDE domains. We built up expertise in diverse structural approaches by studying the relatively tractable association of histones with chaperone proteins showing how they self-associate (Bowman et al., 2015) and form a series of assemblies with histones (Hammond et al., 2016) that are capable of mediating the handling of intact histone H3-H4 tetramers (Bowman et al., 2011). This equipped us with expertise required to generate a structural model for the enzyme Chd1. Over the last year we have adopted the use of single particle Cryo-EM and this has enables us to generate models of Chd1 in solution and engaged with nucleosomes. A manuscript describing this work is currently in preparation.

We have also studied the functions of chromatin remodelling enzymes taking advantage of modern genomic techniques. We found that combinations of enzymes act with partial redundancy to organise nucleosomes over coding regions (Gkikopolous et al., 2011) and more recently the kinetics of this process (Fennessy and Owen-Hughes 2016). We have transitioned to studying the functions of chromatin remodelling enzymes in human cells (Weichens et al., 2016) showing the enzyme SNF2H acts to direct the organisation of nucleosomes adjacent to the architectural transcription factor CTCF and many other transcriptional regulators.

Peer-reviewed publications and other research outputs
List up to 20 of your most significant peer-reviewed publications or other scholarly contributions and other research outputs, e.g. patents. Please ensure that at least five of these are from the last five years. For 10 of these outputs, you may also provide a statement describing their significance (up to 50 words per output).

*Please give citation in full, including title of paper and all authors**
*(*All authors, unless more than 10, in which case please use 'et al', ensuring that your position as author remains clear.)*

1. Wiechens, N., V. Singh, T. Gkikopoulos, P. Schofield, S. Rocha, and T. Owen-Hughes, *The Chromatin Remodelling Enzymes SNF2H and SNF2L Position Nucleosomes adjacent to CTCF and Other Transcription Factors.* Plos Genetics, 2016, PMC 4809547.

ISWI-related remodelling enzymes are shown to position nucleosomes adjacent to the binding sites for a subset of transcription factors at human gene regulatory elements. These include sites bound by the architectural transcription factor CTCF and in this case the organisation of nucleosomes correlates with CTCF function.

2. Hammond, C.M., R. Sundaramoorthy, M. Larance, A. Lamond, M.A. Stevens, H. El-Mkami, D.G. Norman, and T. Owen-Hughes, *The histone chaperone Vps75 forms multiple oligomeric assemblies capable of mediating exchange between histone H3–H4 tetramers and Asf1–H3–H4 complexes.* Nucleic Acids Research, 2016, doi 10.1093/nar/gkw209 **PMC In**

Process

Vps75 is shown to form complexes with histone H3 and H4 consistent with the stepwise assembly and disassembly of histone H3-H4 tetramers. A low resolution model describing the mode of interaction of histones H3 and H4 with Vps75 is presented and is likely to apply to other NAP-1 fold chaperones.

3. Fennessy, R.T. and T. Owen-Hughes, *Establishment of a promoter-based chromatin architecture on recently replicated DNA can accommodate variable inter-nucleosome spacing.* Nucleic Acids Research, 2016, doi 10.1093/nar/gkw331 **PMC In Process**

This study shows that following DNA replication nucleosomes are realigned with promoters with a half-time of 2 minutes. It also showed that the spacing between nucleosomes increases when histone supply is reduced. This challenges some of the models currently used to explain how nucleosomes are positioned across genomes.

4. Gierlinski, M., C. Cole, P. Schofield, N.J. Schurch, A. Sherstnev, V. Singh, N. Wrobel, K. Gharbi, G. Simpson, T. Owen-Hughes, M. Blaxter, and G.J. Barton, *Statistical models for RNA-seq data derived from a two-condition 48-replicate experiment.* Bioinformatics, 2015, PMC 4754627.

5. Bowman, A., C.M. Hammond, A. Stirling, R. Ward, W.F. Shang, H. El-Mkami, D.A. Robinson, D.I. Svergun, D.G. Norman, and T. Owen-Hughes, *The histone chaperones Vps75 and Nap1 form ring-like, tetrameric structures in solution.* Nucleic Acids Research, 2014, PMC 4027167.

6. Ryan, D.P., R. Sundaramoorthy, D. Martin, V. Singh, and T. Owen-Hughes, *The DNA-binding domain of the Chd1 chromatin-remodelling enzyme contains SANT and SLIDE domains.* Embo Journal, 2011, PMC 3155300.

The crystal structure of the C-terminus of the Chd1 protein was determined. This showed that it is a DNA binding domain comprised of SANT and SLIDE domains related to those present in ISWI proteins.

7. Gkikopoulos, T., P. Schofield, V. Singh, M. Pinskaya, J. Mellor, M. Smolle, J.L. Workman, G.J. Barton, and T. Owen-Hughes, *A role for Snf2-related nucleosome-spacing enzymes in genome-wide nucleosome organization.* Science, 2011, PMC 3428865.

This study shows that ATP-dependent chromatin remodelling enzymes act with partial redundancy to organise nucleosomes over coding regions. This is strong evidence that nucleosome positioning in coding regions is directed by enzymes rather than the underlying DNA sequence.

8. Gkikopoulos, T., V. Singh, K. Tsui, S. Awad, M.J. Renshaw, P. Scholfield, G.J. Barton, C. Nislow, T.U. Tanaka, and T. Owen-Hughes, *The SWI/SNF complex acts to constrain distribution of the centromeric histone variant Cse4.* Embo Journal, 2011, PMC 3098484.

9. Bowman, A., R. Ward, H. El-Mkami, T. Owen-Hughes, and D.G. Norman, *Probing the (H3-H4)(2) histone tetramer structure using pulsed EPR spectroscopy combined with site-directed spin labelling.* Nucleic Acids Research, 2010, PMC 2810997.

10. Engeholm, M., M. de Jager, A. Flaus, R. Brenk, J. van Noort, and T. Owen-Hughes, *Nucleosomes can invade DNA territories occupied by their neighbors.* Nature Structural & Molecular Biology, 2009, PMC 2675935.

11. Ahel, D., Z. Horejsi, N. Wiechens, S.E. Polo, E. Garcia-Wilson, I. Ahel, H. Flynn, M. Skehel, S.C. West, S.P. Jackson, T. Owen-Hughes, and S.J. Boulton, *Poly(ADP-ribose)-Dependent Regulation of DNA Repair by the Chromatin Remodeling Enzyme ALC1.* Science, 2009, PMC

3443743.

12. Stockdale, C., A. Flaus, H. Ferreira, and T. Owen-Hughes, *Analysis of nucleosome repositioning by yeast ISWI and Chd1 chromatin remodeling complexes.* Journal of Biological Chemistry, 2006, PMC 1764501.

13. Lia, G., E. Praly, H. Ferreira, C. Stockdale, Y.C. Tse-Dinh, D. Dunlap, V. Croquette, D. Bensimon, and T. Owen-Hughes, *Direct observation of DNA distortion by the RSC complex.* Molecular Cell, 2006, PMC 3443744.

Distortion of single DNA molecules resulting from the action of the RSC complex was followed in real time. Transient reductions in DNA supercoiling were observed and have since been observed in other systems.

14. Flaus, A., D.M.A. Martin, G.J. Barton, and T. Owen-Hughes, *Identification of multiple distinct Snf2 subfamilies with conserved structural motifs.* Nucleic Acids Research, 2006, PMC 4162295.

Snf2 related proteins are classified into 24 subfamilies based on sequence homology within their ATPase domains. The sequence features within subfamilies are mapped to the structures of related ATPases and often found to correlate with distinct biochemical activities. Many subfamilies are broadly conserved in evolution, suggesting distinct functions.

15. Flaus, A., C. Rencurel, H. Ferreira, N. Wiechens, and T. Owen-Hughes, *Sin mutations alter inherent nucleosome mobility.* Embo J, 2004, PMC 1271755.

16. Whitehouse, I., C. Stockdale, A. Flaus, M.D. Szczelkun, and T. Owen-Hughes, *Evidence for DNA translocation by the ISWI chromatin-remodeling enzyme.* Mol Cell Biol, 2003, PMC 12612068.

17. Flaus, A. and T. Owen-Hughes, *Dynamic properties of nucleosomes during thermal and ATP-driven mobilization.* Mol Cell Biol, 2003, PMC 207611.

18. Bruno, M., A. Flaus, C. Stockdale, C. Rencurel, H. Ferreira, and T. Owen-Hughes, *Histone H2A/H2B dimer exchange by ATP-dependent chromatin remodeling activities.* Mol Cell, 2003, PMC 3428624.

At the time of this study chromatin remodelling was considered to involve only DNA distortion. This study showed that the integrity of the histone octamer could be compromised and raises the possibility that remodelling involves reconfiguring the octamer as well as DNA.

19. Havas, K., A. Flaus, M. Phelan, R. Kingston, P.A. Wade, D.M. Lilley, and T. Owen-Hughes, *Generation of superhelical torsion by ATP-dependent chromatin remodeling activities.* Cell, 2000.

Cruciform extrusion was used to show that representative classes of Snf2 related enzymes could generate superhelical torsion in DNA. This provided a direct readout of enzyme activity that did not involve histones. The best explanation for these observations was that superhelical torsion was generated as a result of DNA translocation.

20. Whitehouse, I., A. Flaus, B.R. Cairns, M.F. White, J.L. Workman, and T. Owen-Hughes, *Nucleosome mobilization catalysed by the yeast SWI/SNF complex.* Nature, 1999,

This study showed that one of the ways that the SWI/SNF complex acts to alter nucleosomes is by changing the positions that nucleosomes occupy on the same DNA fragments. A bias was observed for non-dissociative redistribution, but at higher enzyme concentrations dissociative processes were also observed.

Total number of peer-reviewed publications which you have authored/co-authored. Please exclude abstracts and literature reviews.	60

Current research funding (including Wellcome Trust grants)
Please list all active grants only (starting with the most recently awarded). State the name of the awarding body, name(s) of grantholder(s), title of project, amounts awarded, your role in the project, and start and end dates of support. Indicate the number of hours per week that are spent on each project.

Wellcome Trust Senior Fellowship, Tom Owen-Hughes, "Mechanisms for Remodelling Chromatin" £2.1M, Applicant and Principle Investigator, Jan 2012-Dec 2016. 20 hours per week.

Wellcome Trust, Angus Lamond, Julian Blow, Tom Owen-Hughes. “TechnologiesPlatform”. £5,440,234. Co-applicant. Jan 2013-Dec 2017. 2hrs per week.

Please describe how the currently active grants listed above relate to this application

Single particle cryo-EM will represent a key component of the work funded under my current Senior Fellowship. Following on from this we will investigate how alterations to the human SWI/SNF complex contribute to cancers and cryo-EM will be essential for this. The availability of more time on a relatively local microscope will be transformative in comparison to the occasional time we currently obtain in Leiden and Harwell.

5

Applicant	
Full Name	Dr Atlanta Cook
Department	Wellcome Trust Centre for Cell Biology
Division	
Organisation	University of Edinburgh
Address Line 1	Max Born Crescent
City/Town	Edinburgh
Postcode	EH9 3BF
Country	United Kingdom
Telephone No.	0131 650 4995
Email Address	atlanta.cook@ed.ac.uk

Career history (current/most recent first)

From	**To**	**Position**	**Organisation**
10/2016	09/2021	Wellcome Trust Senior Research Fellow	University of Edinburgh
01/2011	09/2016	MRC Career Development Fellow	University of Edinburgh
10/2007	12/2010	Postodoctoral Fellow	MPI for Biochemistry, Martinsried
01/2004	08/2007	Postodoctoral Fellow	EMBL Heidelberg

Career history (current/most recent first)			
From	**To**	**Position**	**Organisation**
10/2003	12/2003	Postdoctoral research assistant	University of Oxford

Education/training				
From	**To**	**Qualification**	**Subject**	**Organisation**
10/1999	09/2003	Doctor of Philosophy (PhD;DPhil)	Biochemistry (Structural Studies on the Catalytic and Regulatory Mechanisms of Protein Kinases)	University of Oxford
10/1995	06/1999	Master of Science (MSc)	Biochemistry	University of Oxford

Source(s) of personal salary support

Wellcome Trust Senior Research Fellowship

Details of any relevant previous experience of managing a resource, a technology or equipment.

I set up a crystallisation facility at the University of Edinburgh in 2012 that is currently used by around 15 people.

Career contributions
What are your most important research-related contributions to date? These may include contributions to health policy or practice, or to technology or product discovery and development.

As a postdoc, working on the structural basis of nuclear export, I solved the structure of the tRNA export factor, Xpo-t in its nuclear (cargo-bound) and cytoplasmic (cargo-free) states. This work revealed how nuclear export factors recognise RNA through shape and charge complementarity, using similar mechanisms to how they recognise proteins. These structures, and the structure of the cytoplasmic form of another export factor, Cse1, allowed us to answer a major question that had eluded the export field for 10 years: the conformational changes that these proteins undergo on cargo release prevents retrograde transport and is the basis for the directionality of transport.

My current research focus is on RNA metabolism in eukaryotes. Our work on nuclear factor 90 (NF90), an RNA binding protein and its binding partner, NF45, revealed structural conservation of these proteins with RNA modifying enzymes. In collaboration with Ulrike Kutay (ETH) we showed that NF90 is involved in ribosome biogenesis and nucleolar architecture. Our recent work, on NF90-dsRNA interactions reveals a mode of binding conserved with ADAR2, an enzyme that catalyses adenosine to inosine editing.

My group has solved a crystal structure of an essential ribosome biogenesis factors, Tsr1, a putative GTPase with an enigmatic role in the late stages of maturation of pre-40S ribosomal particles. Our work reveals that Tsr1 is a molecular mimic of translational GTPases but lacks catalytic activity. This suggests that its primary role in pre-40S particles is to prevent premature association with mature 60S particles in the cytoplasm. Our work also suggests that Tsr1 dissociation is likely to occur prior to (rather than during) a translation-like quality control step in 40S subunit maturation. This allows us to place events that occur on the pre-ribosomal particle in a

temporal context.

To build on these achievements, I am keen to use cryoEM to determine high resolution structures. I have therefore been at the forefront of Edinburgh University's involvement with this proposal.

Peer-reviewed publications and other research outputs
List up to 20 of your most significant peer-reviewed publications or other scholarly contributions and other research outputs, e.g. patents. Please ensure that at least five of these are from the last five years. For 10 of these outputs, you may also provide a statement describing their significance (up to 50 words per output).

*Please give citation in full, including title of paper and all authors**
*(*All authors, unless more than 10, in which case please use 'et al', ensuring that your position as author remains clear.)*

McCaughan U.M., Jayachandran U., Shchepachev V., Chen Z.A., Rappsilber J., Tollervey D., **Cook A.G**. (2016) Pre-40S ribosome biogenesis factor Tsr1 is an inactive structural mimic of translational GTPases. Nat Commun. 7:11789. doi: 10.1038/ncomms11789.

Jayachandran U., Grey H. and **Cook A.G.** (2015) Nuclear Factor 90 uses an ADAR2-like binding mode to recognize specific bases in dsRNA *Nucl. Acids Res.* doi: 10.1093/nar/gkv1508

Wandrey, F., Montellese, C. Koós, K., Badertscher, L., Bammert, L., **Cook, A.G.,** Zemp, I., Horvath, P. and Kutay U. (2015) The NF45/NF90 heterodimer contributes to the biogenesis of 60S ribosomal subunits and influences nucleolar morphology. *Mol. Cell. Biol.* doi: 10.1128/MCB.00306-15

Hector R.D., Burlacu E., Aitken S., Le Bihan T., Tuijtel M., Zaplatina A., **Cook, A.G.** and Granneman S. (2014) Snapshots of pre-rRNA structural flexibility reveal eukaryotic 40S assembly dynamics at nucleotide resolution *Nucl. Acids Res*. doi: 10.1093/nar/gku815

Wolkowicz, U.M. and **Cook, A.G.**, (2012) NF45 dimerizes with NF90, Zfr and SPNR via a conserved domain that has a nucleotidyltransferase fold. *Nucl. Acids Res.* 40:9356-68

Zich, J., Sochaj, A.M., Syred, H.M., Milne, L., **Cook, A.G.,** Ohkura, H., Rappsilber, J., and Hardwick, K.G. (2012). Kinase activity of fission yeast Mph1 is required for Mad2 and Mad3 to stably bind the anaphase promoting complex. *Curr Biol 22*, 296-301.

Bono, F., **Cook, A.G.,** Grunwald, M., Ebert, J., and Conti, E. (2010). Nuclear import mechanism of the EJC component Mago-Y14 revealed by structural studies of importin 13. *Mol Cell 37*, 211-222.

Cook A.G. and Conti, E. (2010) Nuclear export complexes in the frame *Curr. Op. Struct. Biol*. 20:247-52 (Review)

Cook, A.G., Fukuhara, N., Jinek, M., and Conti, E. (2009). Structures of the tRNA export factor in the nuclear and cytosolic states. *Nature* 461, 60-65. (Four F1000 recommendations)

Cook, A., Bono, F., Jinek, M. & Conti, E. (2007) Structural biology of nucleocytoplasmic transport *Ann. Rev. Biochem.* 76:647-71 (Review)

Cook, A. and Conti, E. (2006) Dicer measures up *NSMB* 13:190-2??(News and Views)

Cook, A., Fernandez, E., Lindner, D., Ebert, J., Schlenstedt, G. & Conti, E. (2005) The structure of the nuclear export receptor Cse1 in its cytosolic state reveals a closed conformation incompatible with cargo binding *Mol. Cell.* 18:355-67 (F1000 recommendation)

Cook, A.G., Johnson, L.N. and McDonnell, J.M. (2005) Structural characterization of Ca2+/CaM in complex with the phosphorylase kinase PhK5 peptide. *FEBS Journal*. 272:1511-22

Honda, R., Lowe, E.D., Dubinina, E., Skamnaki, V., **Cook A.,** Brown, N.R. and Johnson, L.N. (2005) The structure of cyclin E1/CDK2: implications forCDK2 activation and CDK2-independent roles. *EMBO J.* 24:452-63

Cook, A., Lowe, E.D., Chrysina, E.D., Skamnaki, V.T., Oikonomakos, N.G. and Johnson, L.N. (2002) Structural studies on phospho-CDK2/cyclin A bound to nitrate, a transition state analogue: implications for the protein kinase mechanism. *Biochemistry*. 41:7301-11

Ghosh, M., Meerts, I.A., **Cook, A.,** Bergman, A., Brouwer, A. and Johnson, L.N. (2000) Structure of human transthyretin complexed with bromophenols: a new mode of binding *Acta Crystallographica D* 56:1085-95

Total number of peer-reviewed publications which you have authored/co-authored. Please exclude abstracts and literature reviews.	13

Current research funding (including Wellcome Trust grants)
Please list all active grants only (starting with the most recently awarded). State the name of the awarding body, name(s) of grantholder(s), title of project, amounts awarded, your role in the project, and start and end dates of support. Indicate the number of hours per week that are spent on each project.

01.10.2016- 30.09.2021 £1.6M Wellcome Trust Senior Research Fellow, ref: 200898/Z/16/Z "Towards a mechanistic understanding of RNA processing machines"

Please describe how the currently active grants listed above relate to this application

RNA editing in trypasosomal parasites is a complex process that is essential for their survival during infection. My SRF work will address how RNA editing enzymes recognise and process editing sites and how the small guide RNAs that are required for this process are generated. To address these processes I will use a combination of structural biology techniques. Guide RNA processing occurs in large protein complexes and single particle cryoEM methods will be essential to answering these biological questions by allowing me to generate close to atomic resolution structural data.

5. Collaborators

Will you require any key collaborators for this proposal?	Yes

Please list any key collaborators* (name and organisation) and provide a very brief outline of their role in the proposed activity.
**The collaborators named may be replaced with suitable alternatives should it be necessary or appropriate to do so.*

Wellcome Trust funded researchers, including those based in the Wellcome Trust Centre for Cell Biology, (Edinburgh) and the Wellcome Trust Centre for Molecular Parasitology (Glasgow) as well as numerous senior fellows are among the applicants and collaborators that will benefit from the foundation of SMIC. Access to state of the art automated cryoEM capability for both macromolecular structure determination and cellular tomography will bring about a transformational change in these investigators capacity to investigate the fundamental processes of life and disease.

The following investigators will use SMIC. Many have contributed project outlines in Section 8 - Details of resource, technology development or equipment. Two-page CVs for each collaborator are also presented. These include brief statements of research interests and how the requested resources will benefit the investigator's work.

Wendy Bickmore - MRC Human Genetics Unit - University of Edinburgh

Christopher Boutell - College of Medical, Veterinary and Life Sciences, University of Glasgow

William Charles Earnshaw - Wellcome Trust Centre for Cell Biology, University of Edinburgh (Wellcome Trust Principal Research Fellow)

Nick Gilbert - MRC Human Genetics Unit - University of Edinburgh

Tracey Gloster - University of St Andrews (Wellcome Trust Research Career Development Fellow)

Gerry Graham - University of Glasgow (Wellcome Trust Senior Investigator)

Patrick Heun - Wellcome Trust Centre for Cell Biology, University of Edinburgh (Wellcome Trust Senior Research Fellow)

Paul Hoskisson - University of Strathclyde

William N Hunter - University of Dundee

Shehab Ismail - CRUK Beatson Institute - University of Glasgow

Arockia Jeyaprakash Arulanandam - Wellcome Trust Centre for Cell Biology University of Edinburgh (Wellcome Trust Career Development Fellow)

Rick Lewis - University of Newcastle

David Lilley - University of Dundee

John M. Lucocq - University of St Andrews

Stuart A. MacNeill - University of St Andrews

Matthias Marti - Wellcome Trust Centre for Molecular Parasitology, University of Glasgow

Jon Marles-Wright - University of Newcastle

Richard McCulloch - Wellcome Trust Centre for Molecular Parasitology, University of Glasgow

Fabio Nudelman - School of Chemistry, University of Edinburgh

Christos Pliotas - University of St Andrews

Julia Richardson - University of Edinburgh

Lilach Sheiner - Wellcome Trust Centre for Molecular Parasitology, University of Glasgow

Steve Sinkins - College of Medical, Veterinary and Life Sciences, University of Glasgow (Wellcome Trust Senior Fellow)

David Tollervey - Wellcome Trust Centre for Cell Biology, University of Edinburgh (Wellcome Trust Principal Research Fellow)

Philipp Voigt - Wellcome Trust Centre for Cell Biology, University of Edinburgh (Sir Henry Dale Fellow)

Helen Walden - University of Dundee

Malcolm Walkinshaw - University of Edinburgh

Julie Welburn - Wellcome Trust Centre for Cell Biology, University of Edinburgh

Malcolm White - St Andrews University

The following collaborators will assist with management of the facility and training.

Martin Wynn - STFC Lead investigator of the CCP-EM project. We shall work closely with CCP-EM to deliver training in computational 3D reconstruction methods.

I confirm that the collaborators named above have agreed to be involved, as described, in the proposed activity and are willing for their details to be included as part of this application.
Confirmed

6. Related applications

Is this or a similar application for funding currently under consideration elsewhere?	No

Is this a resubmission of an application submitted to the Trust within the last 24 months?	No

7. Summary of proposal

Proposal summary
Please provide a summary of your proposal, including key goals, for an expert audience

Cryogenic transmission electron microscopy is revolutionising the field of structural biology, allowing researchers to determine rapidly the structures of macromolecular assemblies at close to atomic resolution. Furthermore, developments in cryogenic imaging of the cell are begining to realise the exciting prospect of in-situ structure determination.
We propose to establish a world-class centre for CryoEM with the primary objective of supporting macromolecular structure determination for life-science researchers in Scotland and northern England. Based in the Medical Research Council - University of Glasgow Centre for Virus Research (CVR), the Scottish Macromolecular Imaging Centre (SMIC) will be equipped with a 300 keV automated cryo-transmission electron microscope that is fitted with a direct electron detection camera, an energy filter and phase plates for high-contrast imaging. The centre will be founded by a consortium of Scottish Universities and serve as the hub for a network of structural biology researchers, fostering collaborations, training programmes and mutual support. Existing cryoEM resources at Glasgow, Dundee and Edinburgh will act as feeder sites with expert technical support

to facilitate project development before presentation at SMIC. Oversight of SMIC by a management group will ensure that highest quality outputs are achieved and that the facility is operated with maximum efficiency.

Lay summary
Please provide a summary of your proposal, including key goals, for a non-expert audience

To understand the processes of life and disease, it is important to understand the shapes of biological molecules. Watson and Crick's ground-breaking work to understand the structure of DNA revolutionised our knowledge of the mechanisms by which traits are passed from parent to child in the form of genes.
Scotland has a thriving community of structural biologists engaged in research to understand the shapes of protein and DNA molecules, their interactions and functions. A powerful new technique has emerged in the past few years; cryogenic electron microscopy (CryoEM). This allows scientists to record images of assemblies, such as viruses or enzymes - the molecular machines that perform vital tasks within the cell. These images can be processed in the computer to determine the structure of the objects at the atomic level. These structures then inform our understanding of how biological processes occur.
The Scottish Macromolecular Imaging Centre is a world-class cryoEM research facility, founded by a partnership of Scottish Universities - Glasgow, Edinburgh, Dundee and St. Andrews. It acts as a hub for a network of expert scientists, fostering new collaborations and training programmes to ensure scientific excellence and a new generation of highly skilled young structural biologists.

8. Details of resource, technology development or equipment

Please describe (i) the equipment requested including a scientific justification for the type and model; (ii) details of the research projects that will benefit from the equipment, how they are funded, and how the equipment will enhance the scientific outputs of the projects; (iii) details of similar equipment in the applicants' departments and adjacent departments and the reasons why it cannot be used for the purposes described; (iv) details of others who may benefit from the requested equipment; (v) the regional, national (and international, if appropriate), context of the application, including any joint funding arrangements; (vi) any relevant background information.

Prof Dame Anna Dominiczak
Vice Principal & Head of College
MVLS College Office
Wolfson Medical School Building
University Avenue
Glasgow G12 8QQ

5 October 2016

Dear Prof Dominiczak

This is to confirm that the Medical Research Council will be happy to provide £400,000 in support of the "Scottish Macromolecular Imaging Centre -SMIC" proposal led by Dr David Bhella at the MRC-University of Glasgow Centre for Virus Research. This contribution would be towards capital support only, as part of a submission to the Wellcome Trust Cryo-Electron Microscopy Equipment Grant call.

Best wishes

Yours sincerely

Dr Declan Mulkeen
Chief Science Officer

Tel: 0207 395 2300
Mobile: 07901 515166
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Dr David Bhella
MRC-University of Glasgow Centre for Virus Research
Sir Michael Stoker Building Garscube Campus
464 Bearsden Road
Glasgow G61 1QH

Glasgow 5th October 2016

Dear David,

SULSA Support for "The Scottish Macromolecular Imaging Centre – SMIC"

I am writing on behalf of the Scottish Universities Life Sciences Alliance (SULSA) to outline our strong support for your bid to develop a CryoElectron Microscopy centred imaging centre in Scotland. SULSA, founded in 2008, represents the Biological Sciences communities from Scotland's principle Universities involved in biological research (Aberdeen, Dundee, Edinburgh, Glasgow, St. Andrews and Strathclyde, with invitations to extend membership to other key Institutions pending). It is our explicit aim to help assure that the collective expertise in research into the Life Sciences here is harnessed in ways to enable sustained scientific outputs at the highest international level. Bringing together multiple Universities in a single bid, such as this one, is central to our aims.

SULSA has been instrumental in supporting numerous facilities now serving scientists across Scotland and beyond, including The National Phenotypic Screening Centre in Dundee, the European Lead Factory at BioCity Scotland, the Scottish Metabolomics facility in Glasgow, Edinburgh Genomics and Glasgow Polyomics and several Imaging platforms across Scotland. A review of equipment requirements conducted by SULSA on behalf of the Scottish Funding Council in 2015 identified the development of CryoElectron microscopy as the most pressing single development that would help build research capability in Scotland. The Wellcome Trust's scheme to build CryoElectron Microscopy represents an excellent opportunity to offer Scotland's scientists access to state of the art equipment that will underpin diverse research activity in structural biology. SULSA, and its partner Universities, support your application with unfettered enthusiasm.

We are delighted also to be able to offer £60,000 to support the bid and will also be able to share our experiences in user access and a sustainability plan.

Yours Sincerely

Professor M. P Barrett (Director)

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School of Biological Sciences

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Web: www.biology.ed.ac.uk

10 October 2016

To whom it may concern

Dear Sir/Madam,

Cryo-Electron Microscopy Equipment grant application (Ref: UNS33184)
The Scottish Macromolecular Imaging Centre (SMIC)

I am writing in support of the bid from the Scottish Macromolecular Imaging Centre (SMIC), a consortium of Scottish universities with a vision to create a world leading cryo-electron microscopy network to support structural biology. We consider access to high-end cryoEM facilities to be essential to our research and lend our strongest support to this application.

The University of Edinburgh has a thriving structural biology community with key focuses on cell division, chromatin biology, RNA metabolism, parasite infection and cancer research. The projects detailed in the scientific case represent a small fraction of research activities in structural biology in Edinburgh. In particular, this bid will facilitate the highest quality data and research output from a number of Wellcome Trust-funded groups, centres and facilities and so represents substantial added value. These include our in-house cryoEM facility (FEI F20 200keV field emission gun with 8Kx8K CMOS camera, cold stage and vitribot, WT087658) the Wellcome Trust Centre for Cell Biology and the Centre for Translational and Chemical Biology (CTCB), which has been supported by a number of multiuser equipment grants.

Within the Wellcome Trust Centre for Cell Biology (WTCCB), two structural biologists, Atlanta Cook and Arockia Jeyaprakash Arulanandam have recently been awarded WT Senior Research Fellowships to support their research programmes on trypanosomal RNA editing and on centromere maintenance and kinetochore function, respectively. Both of these research programmes will make use of single particle cryoEM methods. There is a strong spirit of collaboration within WTCCB and throughout the School of Biological Sciences (SBS). Due to the recent developments in cryoEM, there is a huge appetite across our School to use this technology to solve fundamental problems in chromatin structure and remodelling (Earnshaw, Arulandanam, Allshire, Bird, Stancheva, Richardson, Heun and Voigt groups), RNA processing (Tollervey, Schnaufer and Cook) and cell division (Welburn, Hardwick, Arulandanam, Walkinshaw). Beyond the SBS, researchers in the School of Chemistry will make use of cryoEM facilities to understand biological mineralization (Nudelmann) and the immunobiology of the complement system (Barlow). SMIC will also support cross university collaborations with the School of Medicine on RNA binding proteins involved in chromatin structure (Bickmore, Gilbert, Cook). With access to excellent protein production and

biophysical characterization facilities (housed in the CTCB) and our in-house cryoEM facilities, we are well placed to make optimal use of SMIC facilities, which will be only an hour away in Glasgow.

SMIC also represents an important strategic investment for the University of Edinburgh. Our long-term goal to "Build a New Biology" focuses on excellent infrastructure, technology, training and interdisciplinarity. We are currently recruiting a Reader in Structural Biology with a focus on single particle cryoEM. The unprecedented cooperation within the SMIC consortium will provide an exceptional platform for training a future generation of structural biologists with expertise in single particle analysis and cryoelectron tomography. All members of the network have made both capital and in-kind commitments to this proposal. Our commitments are as follows:

- a cash contribution of £100k towards the capital purchase of the Titan Krios 300keV instrument

- A commitment to 0.5 FTE technical support for the feeder instrument at UoE - £115k over the five years of the award.

- A commitment to support the maintenance cost of the feeder instrument over five years of the award, to the value of £200k in total.

This comes to a total commitment of £415k over the five years of the award. We will also underwrite the annual institutional subscription costs for users at the University of Edinburgh to SMIC, to ensure that the facility operates sustainably and access/throughput is maximized.

In addition to this we have a longer-term commitment to improving and upgrading the cryoEM feeder facility in Edinburgh, with a view to improving data quality and throughput.

Yours faithfully

Professor David Gray, FRSE, FRSB
Head of School

cc: Jayne Glendinning / Caroline Scotland, SBS Research Support Administration
File

Prof J Julian Blow FRSE FMedSci
Dean, School of Life Sciences
University of Dundee
Dundee DD1 5EH, Scotland, UK

PA Mrs Claire Kadoch | c.kadoch@dundee.ac.uk | +44 (0)1382 381042

11 October 2016

To whom it may concern

I am writing in support of the bid to establish The Scottish Macromolecular Imaging Centre (SMIC). Recent advances in cryo electron microscopy have had a transformative effect on many aspects of structural biology and cell biology. This means that any substantial body of research in the area of Life Sciences now requires access to high end facilities in order to be most effective.

While the work of four Dundee research groups is mentioned in the proposal I am convinced this represents the tip of the iceberg in terms of future demand. There are many other groups with interests in the application of cryo-EM both for single particle analysis and tomography. These include additional members of the MRC protein phosphorylation and ubiquitylation unit in Dundee, Yogesh Kalathu (Ubiquitin recognition) and Saptal Virdee (Ubiquitin signalling). The School of Life Sciences in Dundee hosts the Drug Discovery Unit that provides a pharma grade pipeline for compound screening. Historically many of the target proteins have been selected based on their small size and the availability of good structural information. However, the ability of cryo-EM to provide high resolution structural information for larger molecules and complexes presents new opportunities. The group of Alessio Ciulli (ERC investigator - Cullin ligases, chromatin readers) is especially keen to adopt this approach now that we have established an in house capability. Similarly, the groups of Ron Hay (Wellcome Investigator – Ubiquitin and Sumo modification) and Daan Van Alten (Wellcome Investigator – Protein O-GlcNAc modification) have expertise in structural biology and a desire to adopt cryo-EM. In addition, many groups currently using molecular approaches have research questions that are suited to study using cryo-EM. These include the groups of Tracy Palmer (Wellcome Investigator – protein transport), Sarah Coulthurst (Wellcome Senior Fellow – protein secretion), Helge Dorfmueller (Wellcome Dale Fellow – cell wall biogenesis in Streptococci), Tomo Tanaka (Wellcome Principle Fellow – kinetochore function), Julian Blow (Wellcome Investigator – DNA replication). The power of single particle analysis is in part driven by the the development of new computational approaches. In Dundee several members of the Division of Computational Biology have recognised the value in applying molecular modelling to cryo-EM datasets (Ulrich Zachariae, Andrei Pisliakov, Rastko Sknepnek). In addition to single particle analysis recent developments in tomography enable subcellular structures to be studied with unprecedented resolution. Mike Ferguson is interested in applying this approach to study trypanosome surface glycoproteins and Tomo Tanaka to study kinetochore microtubule interactions. Finally, some of the candidates we are considering as future recruits to the school have a requirement for access to cryo-EM. In summary, the demand for cryo-EM goes beyond the four projects mentioned in the application and will add value to a large volume of research including a number of Wellcome Trust Funded programmes.

The four research groups that have adopted the use of cryo-EM over the last two years have been assisted through co-operation with partners in the consortium especially in Glasgow and Edinburgh. Realising the need for research groups to be able to prepare and test the quality of samples in house we have established a feeder facility in Dundee. This includes a Vitroblot system for grid preparation, and a 200 keV Jeol 2010 FEG microscope equipped with at 4k CCD camera. The commitment to establish this facility and the staff time to run it provides an indication of our conviction that this is a necessity for our research going forward. It is a vision of the consortium to strengthen the capability of the feeder facilities in Edinburgh and Dundee over time, but at present access to a 300 keV system equipped with a state of the art detector is rate limiting.

With demand increasing, existing national and international facilities are unable to host all suitable projects in a timely fashion. Frozen grids are transportable and in principle can be shipped to any facility worldwide. However, in practice researchers on specific projects, especially those involving molecules in the lower end of the molecular weight range suited for single particle analysis are often most effective if they are present during collection together with facility staff. For this reason it will be an advantage to all the partner institutions to have access to a facility based in Scotland. Building, on the spirt of co-operation established in training many of the groups that have provided preliminary data, the partner institutions seek to establish a shared facility in Glasgow. The co-operation between the partners is unprecedented with St Andrews, Edinburgh and Dundee committing significant capital towards the facility to be located in Glasgow. The commitment of matching capital from Dundee is £125,000 paid in annual instalments of £25,000. In addition, all of partners are committing substantially in kind. From Dundee our commitment has been to establish and maintain a feeder facility. This includes a 0.5 FTE position to support use of the feeds facility amounting to £193,018 over the five years of the award. This position will assist in training users to use the microscope and to provide advice and assistance on sample preparation. In addition to serving the needs of researchers in Dundee, this will provide capability for researchers from the University of St Andrews which is located 10 miles to the south. This will provide a supply of high quality samples for submission to the 300 keV facility in Glasgow. We anticipate that the demand from Dundee will amount to at last 24 sessions (48 days per year).

In summary, this application has my full support and I hope it will fulfil an important role in building capability in this important area across all regions of the UK.

Prof J Julian Blow FRSE FMedSci

University of St Andrews | FOUNDED 1413

Office of the Principal
Derek Woollins
Vice-Principal (Research) and Provost

The Wellcome Trust

Oct 10 2016

Dear Committee

Grant Application - Electron Microscopy

St Andrews University is committed to the bid led by Dr David Bhella (Glasgow) for an EM facility to be shared by Glasgow, Edinburgh, Dundee and St Andrews Universities. My colleague Professor James Naismith is an applicant on the grant but in reality he stands for a large number of scientists at St Andrews who see EM as the significant resource for structural biology. St Andrews has invested strongly in medically related biology focussed around infection and the immune response to it. We have world class efforts in virology (led by newly appointed Professor David Evans), parasitology (led by Professor Terry Smith) and bacteriology (led Professor Stephen Gillespie). As the Trust know structural biology is very strong at St Andrews, in addition to Naismith, Professor Garry Taylor (Deputy Principal), Professor Malcolm White (member of EMBO), Dr Tracey Gloster (a Wellcome research fellow), Dr Christos Pliotas (a Royal Society of Edinburgh research fellow), Dr Stuart MacNeil and Dr Uli Schwarz-Linek have enviable reputations in understanding biology at the molecular level. This strength in structural biology has allowed us to link chemistry, biology, medicine and physics in our flagship Biomedical Sciences Research Complex (BSRC). The University of St Andrews has invested its own resources alongside the Wellcome Trust and other sponsors to build this initiative from scratch over the last 16 years. I am sure I do not need to remind you that in proportion to its size, St Andrews has invested more in biomedical sciences than any other UK university. Whilst we recognise the vital importance of EM, St Andrews itself could not efficiently use a dedicated Krios. Rather, we strongly favour the consortium outlined in the proposal where our scientists get routine access to the lower specification instruments within the grouping to gain the preliminary data necessary to use time on the Krios effectively. This model of a well managed shared facility is much better value for the UK and is in the longer term sustainable.

When the EM bid is funded by the Wellcome Trust St Andrews will contribute £75 K of capital and we will support a 0.5 FTE (£30 K per year) of a senior scientific officer for five years. We are constructing a new data centre and multi node GPU cluster; we will contribute 0.5 peta bytes of so called "hot" storage (estimated value £40K per year) and 10 days of time per year on the cluster (estimated value £5K per year). These facilities will be open to colleagues within the consortium. In total our investment in the EM will be £475K. We have

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agreed that St Andrews will commit to silver membership of the consortium in its first five years, this best reflects the likely demand of scientists from St Andrews.

The four Universities have an established record of cooperation through pooling initiatives, joint grants and shared projects. This history of partnership should give confidence to the Trust that the facility is genuinely pan institution and will be supported.

Yours sincerely

Derek Woollins FRSE FRSC
Vice Principal Research and Provost

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Professor Owen Sansom, Interim Director

6th October 2016

Professor Dame Anna Dominiczak
Vice Prinicipal and Head of College
MVLS College Office
Wolfson Medical School Building
University Avenue
Glasgow G12 8QQ

Dear Anna

With reference to David Bhella's coordinating of a bid to the Wellcome Trust for a new Scottish-Cryo-EM facility which will be located in the Stoker Building on Garscube Campus, I would like to confirm our full support of this proposal.

Recent advances in the development of a new generation of electron microscopes, direct electron detectors, data collection and analysis have transformed single-particle cryo-electron microscopy (cryo-EM) as a method for determination of high-resolution structures of biological macromolecules. Structures of several macromolecules such as eukaryotic ribosome, spliceosome, anaphase-promoting complex have now been determined at near atomic resolution by using these techniques. The Beatson Institute for Cancer Research has strong structural biology teams (Danny Huang's, Shehab Ismail's and Martin Drysdale's groups) and other groups who are interested in structures of various macromolecules. Their works will benefit immensely by this new cryo-EM facility.

We are delighted to contribute the funding for a Grade 6 Research Association position to support the implementation of this very exciting technology in Glasgow.

Yours sincerely

Owen Sansom
Interim Director

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11th October, 2016

Your reference: The Scottish Macromolecular Imaging Centre

Dear David,

I am very happy to provide a warm letter of support for your application to the Wellcome Trust for resources to provide a Cryo-EM facility (The Scottish Macromolecular Imaging Centre (SMIC)) for Scotland, based in Glasgow. This is an exciting proposal that would add significantly to a lot of work done at Aberdeen by multiple investigators - including the work funded via my own Wellcome Trust Investigator Award. Although our university is not able to contribute financially to your application we are happy to endorse the importance and concept of the SMIC. We retain a local capability of cryo (imaging) EM with tomography to which the availability of single-particle data collection would enhance a number of project's. It makes sense to centralise this facility and to encourage equipment and expertise sharing to ensure that maximum benefit accrues from such an investment.

Yours sincerely,

Neil A R Gow

Professor Neil A.R. Gow FRS, FMedSci, FRSE, FSB, FAAM
Director, Aberdeen Fungal Group
Director, Wellcome Trust Strategic Award in Medical Mycology and Fungal Immunology

10 October 2016

Professor JE Calvert FMed Sci
Acting Pro-Vice-Chancellor
Faculty of Medical Sciences
Newcastle University
Medical School
Framlington Place
Newcastle upon Tyne
NE2 4HH

Dear Dr Bhella

I am delighted to write on behalf of the Faculty of Medical Sciences at Newcastle University, to express our strong support for your application to the Wellcome Trust for funds to develop the SMIC cryo-electron microscopy (cryo-EM) facility at the University of Glasgow. Newcastle University scientists Lewis and Marles-Wright are already collaborating with each other and with SMIC applicants Bhella and Spagnolo in their cryo-EM endeavours. It is crucial to the success of these projects that Lewis and Marles-Wright can access high-end cryoEM; we welcome your offer of access to the requested instrument's up-time, the costs for which they will meet from funds requested on current and future grant applications for this purpose.

In recent years Newcastle University has made major investments to embed structural biology as a central research capability for the biosciences. This investment has seen appointments at the Professorial (Lewis, Noble, Endicott, van dan Berg), lecturer (Salgado, Marles-Wright) and Research Fellow (Davies) levels. These appointments, in turn, have been supported by major and sustained capital investment by the university, most recently this year in the form of a £0.9M Research Investment Fund award to provide a next-generation, highly automated Liquid MetalJet™ X-ray diffraction facility. Structural biology is a key part of research at Newcastle University sustained by programmatic support from the Wellcome Trust (Gilbert, Errington, Vollmer), MRC (Endicott, Noble, Lewis), and CRUK (Noble, Endicott). All of this research is focussed on questions of biomedical interest ranging from bacterial cell biology through to oncology.

The Faculty and the University recognise that recent technical advances in cryo-electron microscopy have resulted in a step-change in applicability, throughput and achievable resolution that will play an important role in addressing the exciting biological challenges of the future. The proposed SMIC facility at Glasgow will allow the step up from imaging negatively-stained samples on a Newcastle-based transmission electron microscope, to collecting the cryo-electron micrographs in Glasgow, and/or at the eBIC national facility, that are needed to solve molecular problems at near-atomic resolution. Our current TEM instrument is housed in a state-of-the-art imaging facility that is centrally supported by the University and is used by scientists from disciplines ranging from biomedical sciences to materials. This facility has seen recent investment in high-end scanning electron microscopes, and there is recognition at the Faculty and University level that further enhancement of its entry-level TEM capability, especially in the context of the national EM strategy, is a matter of urgent strategic priority. We therefore anticipate that Newcastle University will update and upgrade its current TEM in further support of the unmet needs of the structural biologists – and other users - with internal funds allocated from the £30M Research Investment Fund.

Yours sincerely

Professor Jane Calvert
Acting Pro-Vice-Chancellor FMed Sci

Professor Nick Wright
Pro-Vice-Chancellor Research and Innovation

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Professor Richard J. Lewis, MA, D.Phil

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Dr David Bhella
Centre for Virus Research,
Sir Michael Stoker Building,
University of Glasgow

4th October 2016

Dear David,

I write in support of your bid to the Wellcome Trust for a 300 keV cryo-transmission electron microscope (cryo-TEM) to establish the Scottish Macromolecular Imaging Centre (SMIC) at the University of Glasgow. Given the well-documented recent advances in cryo-TEM it is imperative that the the traditional strength of UK structural biology is not undermined by a failure to invest in a coherent national strategy for top-flight cryo-TEMs. In my opinion, such a strategy requires the establishment of well-connected regional 'hubs' to support a local critical mass of world-class researchers. The 'hub' must have demonstrable expertise in running and using high-end cryo-TEMs effectively, with a history of collaboration and with the scope to help develop and train researchers from beyond the hub's immediate boundary. There are no cryo-TEMs in Newcastle University (or in the wider region), nor the academic expertise to maintain this sort of technology. It is vital to the long-term success of my group's research to be able to access both the pre-existing 200 and the requested 300 keV cryo-TEMs on a single site that is within easy striking distance of Newcastle. The SMIC would seem to provide the solution to all of these problems.

My group has used cryo-TEM in the past in our studies on the 'stressosome' (Marles-Wright *et al*, 2008, *Science* **322**, 92-6), a 1.8MDa signalling complex that co-ordinates the response of some bacteria to stress. The structure revealed how the proteins assembled into this fascinating molecular machine, but the mechanism of signaling remains unknown, and in 2017 we will commence a new, EC-funded ITN project called 'PATHSENSE' to determine the stressosome's signaling mechanism using the complementary techniques of X-ray crystallography and the single particle analysis, which requires access to cryo-TEM. My group also investigates the biosynthesis of the bacterial cell wall, and how this phenomenon is co-ordinated with cell division. Since some of the key proteins are large (~90kDa), form dimers and higher order complexes with other proteins (e.g. Cleverley *et al*, 2014 *Nat Commun* **5**, 5421; Rismondo *et al* 2016, *Mol Microbiol* **99**, 978-998), they are likely to form interesting and EM-amenable projects that we will work towards generating in the coming months, with a long-term ambition of their structure determination by cryo-TEM in conjunction with the SMIC. To this end, Newcastle University has accepted the need to update and upgrade the existing rather aged non-cryo-TEM that will allow us to work up samples locally in negative stain – and potentially in cryo – before accessing the state-of-the-art 200 and 300 keV instruments at SMIC for full structure determination. This approach will provide the most effective training platform for junior researchers who seek to integrate different experimental approaches to structural biology problems.

Wishing you every success with your application

Dr Jon Marles-Wright
Senior Lecturer
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Newcastle upon Tyne
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Jon.marles-wright1@ncl.ac.uk

6 October 16

Dr David Bhella
Centre for Virus Research
Sir Michael Stoker Building
University of Glasgow

Dear David,

I write in support of your bid to the Wellcome Trust for funds to purchase a 300 keV cryo-transmission electron microscope (cryo-TEM) to establish a Scottish Macromolecular Imaging Centre based in the Centre for Virus Research at the University of Glasgow. In light of the recent advances in cryo-TEM and its growing importance as a tool for structural characterisation of cells and macromolecular complexes it is imperative that researchers working within UK structural biology community have access to state of the art cryo-TEM equipment to allow us to continue to develop our world-leading research in structural biology.

To this end, a strategy for the creation of regional cryo-TEM hubs with a critical mass of structural biology researchers is an approach that will support the development of expertise and capacity across the United Kingdom and allow us to build on our world-class research and become global leaders in the field of structural analysis by cryo-TEM. The foundation of a Scottish Macromolecular Imaging Centre (SMIC) would bring together researchers at hub-institutions in Scotland and the north of England and give access to technical expertise, training and state-of-the art equipment in a purpose built laboratory environment. With your excellent track record of running a cryo-TEM facility and developing methods for imaging difficult macromolecular targets I have no doubt that the proposed SMIC will be a highly successful centre for structural biology and will produce outstanding scientific outputs. Scottish universities have an excellent track record of cooperation through SULSA and SUPA and the proposed structure of SMIC will build on this, with a world-class facility that will cooperatively managed through the partner universities.

I have used cryo-TEM and single particle analysis in my research for the past ten years and have published a number of single-particle reconstructions of macromolecular complexes (Marles-Wright *et al*, 2008, *Science*, **322**, 92-6; Yang *et al*, 2009, *EMBO Reports*, **10**, 997-1002). My current BBSRC funded work on understanding the structure and function of bacterial nanocompartments (BBSRC: BB/N005570/1) will use a combination of biochemical analyses, X-ray crystallography, and cryo-TEM, to determine the mechanism of iron storage and enzyme sequestration within the virus capsid-like shell of the bacterial nanocompartment. As part of this grant we have funding to support access to cryo-EM facilities and hope to collaborate with the SMIC to produce high-resolution reconstructions of bacterial nanocompartments.

To facilitate the pipeline from purified protein complexes to high-resolution cryo-EM reconstructions, the University of Newcastle has acknowledged the need to upgrade their existing transmission electron microscopy facility, which currently houses a twenty year-old Philips CM100 microscope, with a 120 keV instrument to allow screening of samples by negative stain

before accessing the instruments at the SMIC. The upgraded equipment will allow for the provision of local training for junior researchers in sample preparation and image data collection, and maximise the efficiency of our use of any time we have on the 200 and 300 keV SMIC instruments.

I wish you every success with your bid and look forward to working with the SMIC in the future.

Yours Sincerely,

Jon Marles-Wright

Science & Technology Facilities Council
Daresbury Laboratory

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To:
Dr David Bhella
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Scotland (UK)

Dr Martyn Winn
RCaH: 01235 567865
Daresbury: 01925 603455
E-mail martyn.winn@stfc.ac.uk

Date: 6-Oct-2016

Re: The Scottish Macromolecular Imaging Centre, Proposal Number UNS33184

Dear David,

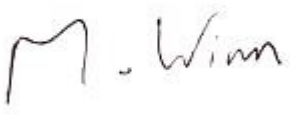
We are writing on behalf of the MRC-funded CCP-EM project (www.ccpem.ac.uk). Over the last four years, CCP-EM has built up a community of users and developers in biological cryoEM. More recently, we have become involved in providing computational support for the new national facility on the Harwell site (eBIC), and have created the first version of a software suite. CCPEM has had its funding renewed for 5 years, and we are now an integral part of the UK infrastructure for cryoEM, as CCP4 is for macromolecular crystallography.

An important part of the remit of CCP-EM is training of the UK community in all computational aspects of cryoEM. To-date, we have run hands-on courses on single particle reconstruction with Relion, model fitting and building, and sub-tomogram averaging. While many of these courses have taken place on the Harwell campus, we aim to support other regions. To this end, we held the latest Relion workshop at Leeds (19th Sept 2016) for 30 students. Following the concept of train-the-trainer, the course was repeated the following day by local tutors for a further set of local scientists. We would be delighted to help organise similar courses at Glasgow. As a minimum, we would help advertise these courses, and could also provide help with course materials, software setup and registration, as required.

As there is continued investment in cryoEM in the coming years, and regional centres are set up or expanded, we also aim to provide computational support where we can. The eBIC centre is pioneering the availability of cryoEM as an automated facility, with streamlined access, data management and software support. CCP-EM is helping with the provision of software for users at eBIC. As a national consortium, it is also our role to help disseminate these advances to regional centres, such as the one in the current proposal. CCP-EM recently presented at a Biochemical Society training day (https://www.biochemistry.org/Events/tabid/379/View/Conference/MeetingNo/td011/MeetingID/2671/Default.aspx) which was designed to inform potential users of the steps required to collect and process data at eBIC. Similar training would be appropriate for regional centres.

In conclusion, CCP-EM recognises the explosion in interest for cryoEM, and aims to support all groups in the UK to maximise the benefits. We thus wholeheartedly support the current proposal, and if successful will work with the new centre to train the next generation of users.

Yours sincerely,



CCP-EM

Martyn Winn
PI for CCP-EM grant

Peter Rosenthal
Crick Institute, Chair of CCP-EM

Neil Ranson
University of Leeds, Deputy Chair of CCP-EM

Does your proposal involve a clinical trial?	No

You may submit up to two A4 pages of additional information (such as graphs, figures, tables and essential unpublished data).

Supporting statement from host organisation
Please upload the supporting statement from your host organisation, signed by an appropriate senior authority, indicating how the request for equipment fits with the overall strategic context of the organisation (referring to any other current multi-user equipment applications submitted to the Trust from your organisation, if appropriate), taking into consideration regional and national contexts if appropriate.

University of Glasgow | College of Medical, Veterinary & Life Sciences

14 October 2016

Dr Tom Collins
Senior Portfolio Developer
The Wellcome Trust
215 Euston Road
London
NW1 2BE

Dear Dr Collins,

The Scottish Macromolecular Imaging Centre

The University of Glasgow is firmly committed to establishing the Scottish Macromolecular Imaging Centre (SMIC) and wholly supportive of this application to the Cryo-Electron Microscopy Equipment call.

Our vision is to create a world-class centre for structural biology that will bring together and further develop the critical mass of expertise from across Scotland and northern England. This vision fits perfectly within our longer term strategic objectives for research and provides vital support for our existing world-class researchers and a number of recent strategic appointments.

With leadership from Dr David Bhella, from the Medical Research Council – University of Glasgow Centre for Virus Research (CVR), our partners have engaged wholeheartedly in this vision and have underlined their support with funding and in-kind contributions amounting to £1.8M. We have also gained emphatic support for bringing this opportunity to Scotland – with the Scottish Funding Council and the Scottish Universities Life Sciences Association contributing £160k and have secured a further £400k from the Medical Research Council, in recognition of the research opportunities arising from this consortium.

The University of Glasgow's commitment to this venture is significant. We are investing £450k to embed SMIC within the CVR and provide our 'gold' membership to the facility and will further invest in two full-time posts to support the sustainability of this vital resource. Together with the CVR's ongoing commitment to support SMIC through maintenance of the current infrastructure and the Bhella research group, this constitutes an immediate commitment of over £1M.

Professor Dame Anna F Dominiczak DBE MD FRCP FAHA FRSE FMedSci
Regius Professor of Medicine
Vice Principal and Head of College of Medical, Veterinary and Life Sciences
Wolfson Medical School Building, University Avenue
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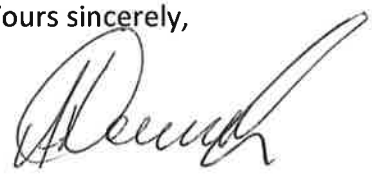
With support from the Wellcome Trust and the commitments from our partners, the Scottish Macromolecular Imaging Centre will position Glasgow at the heart of Scotland's structural biology community and will provide clear opportunities to grow and expand our expertise and capabilities in this area.

I wholeheartedly support this application and the opportunities that the Centre will bring.

Yours sincerely,



ANNA DOMINICZAK

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CVs of additional equipment users
You may attach brief CVs of any additional individuals who will make use of the equipment, and are mentioned in the application.

(Curricula vitae PDF - Combined_CVs_17_10_16.pdf) is included as an appendix within this file.

Key references
You should give the citation in full, including title of paper and all authors.

Applicant key references

Bhella

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Huang

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Are there any papers listed in your 'Key references' section as being "in press" that you wish to submit to us?	No

9. Scientific and technical management

Provide details of: (a) the sharing and management of the equipment, resource or technology development, including how access will be managed and prioritised (if appropriate); (b) any specialist expertise or technical support required and who will provide this; (c) proposed plans for the long-term sustainability of the equipment, resource or technology development.

Scientific and technical management

i) Management of the Scottish Macromolecular Imaging Centre

Led by the University of Glasgow, the Scottish Macromolecular Imaging Centre (SMIC) will provide access to high-end cryomicroscopy for the structural biology communities of Scotland and northern England. A Management Group, chaired by David Bhella (Centre Director) and including representation from each partner will monitor usage and outputs and oversee the resource as it moves towards self-sustainability. The group will meet bi-monthly initially, moving towards a six-monthly schedule once the centre is established. During the installation phase, a local operations group, led by the Centre Director, will progress the project and report to the Management Group.

Membership of the Management Group

Institution	*Representative*
Chair and Facility Director	David Bhella
University of Glasgow	Laura Spagnolo

University of Edinburgh	Atlanta Cook
University of St. Andrew's	James Naismith
University of Dundee	Tom Owen-Hughes
MRC - University of Glasgow Centre for Virus Research	Agnes Simpson (Business Manager)

An External Scientific Advisory Group (ESAG) comprising internationally renowned cryoEM experts will undertake a biennial review of the performance of the facility.

ii) Access to SMIC and future sustainability

Management and scheduling policies for SMIC are modelled on those in place at the UK eBIC facility at Harwell.

Data collection sessions: High-end cryomicroscopes are designed for automated data collection, however specimen screening and set-up for data collection is time-consuming, especially when setting up tomography projects. Sessions will therefore be allocated as 48-hour slots on the assumption that specimens imaged in one 48-hour session will yield sufficient data for close to atomic resolution structure determination by single-particle reconstruction, or many tens of tomograms. Thus, it is envisaged that data collection sessions will be set up every Monday, Wednesday and Friday (the weekend sessions running for up to 72 hours). The microscope will therefore run 24 hours 7 days a week for most of the year.

Block Allocation Groups: Rather than subjecting each application to peer review, microscope time will be allocated to each institution through Block Allocation Groups (BAGs). The institution's BAG-Lead, an expert structural biologist, will have responsibility for assessing preliminary data to ensure that the projects are ready for analysis. Allocation of microscope time will be primarily on the basis of scientific merit within each BAG. Scheduling of projects will be coordinated with the laboratory manager through a shared booking system.

Tiered Subscriptions: The BAG system will work on a tiered-subscription model to ensure continued funding of the facility. Industry will pay commercial rates and academic institutions will benefit from three levels of access:

- Gold, for institutions with a considerable investment in structural biology research and that have made a substantial capital contribution, will allow the buy-out of a substantial number of sessions per year.
- Silver, for institutions with fewer potential users, to opt for a lower level of financial commitment and a commensurate level of access.
- Bronze, will allow access on a pay-as-you-go basis to all institutions including those external to the consortium.

Development and triage of bronze access projects will be undertaken by the laboratory manager with time on the University of Glasgow's CVR, JEOL 2200, being made available for this purpose if

required.

Scheduling will be established quarterly by the laboratory manager, with each institution requesting time allocation according to on-going need. An agreed proportion of microscope time will also be ear-marked for training, applications development and maintenance.

iii) Specialist Expertise and Technical Support

Developing Specialist Expertise: SMIC will be at the centre of an integrated 'hub and spoke' network of structural biologists, linking resources and infrastructure across Scotland and exploiting pre-existing expertise at the University of Glasgow to upskill users and build capacity.

The existing 200 keV cryomicroscopes across the consortium will play an essential role, acting as feeder sites for SMIC, allowing researchers to build their expertise and develop projects locally. Cryomicroscopy requires both highly-skilled, experienced users and considerable investment of time and resource to develop projects to the point that they are suitable for high-resolution structure analysis. The long-term success of the facility will be founded on the development of local expertise across the consortium partners. To enable this, technical support posts (0.5 FTE at each site) will be dedicated to supporting the consortium at Dundee and Edinburgh. A further 0.5 FTE post at St. Andrews will support project development across the consortium and coordinate training programmes.

Our consortium comprises more than 30 investigators actively engaged in research projects that will immediately benefit from SMIC. Many have ongoing projects that have yielded preliminary data, while some are just entering the field. The Bhella and Spagnolo groups in Glasgow have an established reputation and are experienced practitioners of the technique. Owen-Hughes and Nudelman are leading the development of capacity at Dundee and Edinburgh . In the early stages of SMIC, these investigators and SMIC personnel will engage heavily with partners to train researchers, develop projects and build expertise.

Technical staffing: To serve the user community and ensure that the microscope is performing optimally we will appoint two FTE posts at SMIC. These posts are funded by University of Glasgow.

- A laboratory manager (Grade 7-8), reporting to the centre director, will provide expert support, training and project development and oversee the operation and maintenance of the new instrument as well as integrating the new instrument with the existing facilities at the CVR
- An imaging specialist (Grade 6-7), reporting to the laboratory manager, will provide dedicated support to users by loading samples into the instrument and setting up data acquisition sessions as well as overseeing data curation and archive.

It is anticipated that both the laboratory manager and imaging specialist will be experienced cryomicroscopists. Expertise in these areas is available locally and we are confident that highly skilled and dedicated personnel can be appointed to these positions in a timely manner.

iv) Long-term sustainability
Will be achieved through several approaches:

1. Cost recovery: our tiered subscription model will ensure optimum cost recovery and provide a fully sustainable resource over a projected ten to fifteen year operating period. Subscription charges will support consumables (estimated at £60k per year – source FEI) and importantly, will accumulate sufficient funds to prepay a further five years of maintenance and service contract charges by year six. Staff costs will not be recovered and are a component of University of Glasgow's contribution to this proposal.

2. Building capacity through training and mentoring: Ensuring a highly skilled user base will be of paramount importance to the success of this project. We will therefore work together to develop training and mentoring schemes to ensure that we all reap the maximum benefit of our new capability.

To support the learning of researchers entering the field we held our first residential training course at the CVR in January 2016. This was attended by eight post-doctoral and post-graduate scientists from Dundee, Edinburgh, Glasgow and St. Andrew's and was intended to give an introduction to the theory and practice of cryomicroscopy. Such hands-on training courses will be crucially important and we envisage running further introductory courses on an annual basis. We shall also run more advanced courses on specialised areas such as cryotomography, cellular cryomicroscopy and cryomicroscopy of membrane proteins. To help users develop their skills in computational methods, we shall partner with CCP-EM to deliver courses on image processing and three-dimensional image reconstruction (letter of support - section 8).

Close oversight of projects passing through the resource, by the management group, will allow us to identify both successes and challenges. Researchers will then work together to share best-practice and overcome difficulties in work-flows, providing a support network in project development and specimen preparation.

To further share our experiences of working with the latest cutting-edge technology that our facility will provide, we shall hold annual one-day symposia. This will allow researchers to showcase results generated and discuss new innovations in the field. Regular symposia will also provide a platform for users to discuss the management of the facility and highlight any perceived short-comings. We shall seek support from SULSA, industry and learned societies to facilitate these events.

It is vitally important for the UK to maximise the benefits of its investment in cryomicroscopy by working to ensure a ready supply of expert young structural biologists. To this end we intend to seek funding for a RCUK or WT Doctoral Training Partnership in structural biology that will provide opportunities for the brightest young students to work with this exciting technology. The DTP will comprise academics from this consortium and will allow students to access world-class structural biology and biophysical resources and to explore a diverse range of high-profile research projects.

3. Development of new ambitious research collaborations: SMIC will serve as far more than a service to individual research groups. Our shared interest in cryomicroscopy research has led to the foundation of a network of researchers for whom this facility will be a high-value asset. Our shared resources, training and mentoring programmes will foster collaborative links across Scotland, integrating established structural and biophysical research platforms and generating further research income through grants and contracts.

You may attach supporting information demonstrating future sustainability, e.g. extracts from business plans. Details of any proposed management or advisory committees may also be uploaded.

Section 9 – Supporting information

1. ***External advisory board membership***
2. ***SMIC costings statement***
3. ***Sustainability business plan***
4. ***business plan appendix – capacity and partner benefit structure***

External Scientific Advisory Group membership

To provide expert external evaluation and guidance, we have recruited a committee of experts in cryogenic electron microscopy. Every two years a report will be generated, detailing the projects undertaken and resulting outputs. The ESAG will convene in Glasgow to discuss the activities of the consortium and provide advice to ensure that we maximise the benefits of the facility. We will time meetings of the ESAG to coincide with our regular symposia.

ESAG membership:

Helen Saibil
Department of Biological Sciences,
ISMB
Birkbeck College
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Email h.saibil@mail.cryst.bbk.ac.uk

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Francis Crick Avenue
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School of Molecular and Cellular Biology
Email n.a.ranson@leeds.ac.uk

University of Glasgow
Wellcome Trust CryoEM
Costings Statement

1. Capital Costs	**£000**	**Funded by WT**	**Funded by Partners**
CryoEM Purchase	4,300	4,000	300
Room preparation at UoG	230		230
IT Support	200		200
Total initial investment	4,730	4,000	730
2. Operating Costs (10 years)			
Staff	1065		1065
Maintenance (including 5 year cover purchased at end of year 10)	1586		1586
IT Storage (including future expansion purchased at end of year 10)	686		686
Consumables (gasses, energy, spares)	628		628
Training	75		75
Advisory Board	25		25
Total 10 year running costs	4065		4065
Total Combined Facility Costs	**8,795**	**4,000**	**4,795**
Funding Split		**45%**	**55%**

Edinburgh Feeder Microscope Contribution
0.5 FTE technical support 114 (5 year commitment)
Maintenance 200 (5 year commitment)

Dundee Feeder Microscope Contribution
0.5 Staff support 193 (5 year commitment)

St Andrews In Kind
0.5FTE Imaging Specialist 150 (5 year commitment)
Access to 500TB High Performance Data Centre 200 (5 year commitment)
10 days per annum GPU processing capacity 25 (5 year commitment)

Glasgow Feeder Microscope Contribution
Maintenance 344 (5 year commitment)

Glasgow InKind Contribution (Sir Michael Stoker Building Infrastructure) 263 (already incurred)

Capital Investment Proposal
Wellcome Trust CryoEM Facility
Business Plan

	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	
Discount Factor	1.00	0.95	0.90	0.86	0.81	0.77	0.74	0.70	0.66	0.63	0.60	0.57	0.54	0.51	0.49	0.46	0.44	0.42	0.40	0.38	0.36	
1. Capital Investment Requirements																						
Capital Costs £K	**Year 0**	**Year 1**	**Year 2**	**Year 3**	**Year 4**	**Year 5**	**Year 6**	**Year 7**	**Year 8**	**Year 9**	**Year 10**	**Year 11**	**Year 12**	**Year 13**	**Year 14**	**Year 15**	**Year 16**	**Year 17**	**Year 18**	**Year 19**	**Year 20**	**Total 20 Years**
Cryo-EM Purchase	(4,300)																					(4,300)
Installation Costs	(230)																					(230)
IT Infrastructure Costs	(200)			(200)			(220)				(266)											(886)
Total Capital Expenditure £K	**(4,730)**	**0**	**0**	**(200)**	**0**	**0**	**(220)**	**0**	**0**	**0**	**(266)**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**(5,416)**
Capital Grants / Donations £K																						
Wellcome Trust Capital Funding	4,000																					
SFC Capital Funding	100																					
MRC Capital Funding	400																					
St Andrews	75																					
Edinburgh	100																					
Dundee	25	25	25	25	25																	
Glasgow	200																					
Sulsa Contribution	60																					
																						0
Total Capital Grants / Donations £K	**4,960**	**25**	**25**	**25**	**25**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**5,060**
Net Capital Expenditure	**230**	**25**	**25**	**(175)**	**25**	**0**	**(220)**	**0**	**0**	**0**	**(266)**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**(356)**

2. Cashflow Impact on Income & Expenditure																						
	Year 0	**Year 1**	**Year 2**	**Year 3**	**Year 4**	**Year 5**	**Year 6**	**Year 7**	**Year 8**	**Year 9**	**Year 10**	**Year 11**	**Year 12**	**Year 13**	**Year 14**	**Year 15**	**Year 16**	**Year 17**	**Year 18**	**Year 19**	**Year 20**	**Total 20 Years**
Incremental Income																						
																						0
																						0
University of Glasgow 1FTE contribution		57	59	61	63	64	65	66	67	68	69											641
Beatson Institute of Cancer Research 1 FTE contribution		38	39	40	41	42	43	44	45	46	47											423
Income from Gold Subscription Partners to cover running costs (per Billing Appendix)		150	150	150	150	150	150	150	150	150	150											1,500
Income from Silver Subscription Partners to cover running costs (per Billing Appendix)		28	28	28	28	28	28	28	28	28	29											281
Income from Bronze Subscriptions (per Billing Appendix - build up from 25% to 75% capacity over 4 years)		35	49	70	105	105	105	105	105	105	105											889
Total Incremental Income £K	**0**	**308**	**325**	**349**	**387**	**389**	**391**	**393**	**395**	**397**	**400**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**3,734**
Incremental Costs																						
Lab Manager (Grade 8)		(57)	(59)	(61)	(63)	(64)	(65)	(66)	(67)	(68)	(69)											(641)
Research Technician (Grade 6)		(38)	(39)	(40)	(41)	(42)	(43)	(44)	(45)	(46)	(47)											(423)
Maintenance Contracts							(755)				(831)											(1,586)
Consumables		(60)	(61)	(61)	(62)	(62)	(63)	(64)	(64)	(65)	(66)											(628)
Training Costs		(10)	(5)	(10)	(5)	(10)	(5)	(10)	(5)	(10)	(5)											(75)
Scientific Advisory Group Costs			(5)		(5)		(5)		(5)		(5)											(25)
Total Incremental Costs £K	**0**	**(165)**	**(169)**	**(172)**	**(176)**	**(179)**	**(936)**	**(184)**	**(187)**	**(189)**	**(1,022)**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**(3,378)**
Net Surplus / (Deficit)	**0**	**143**	**156**	**177**	**211**	**211**	**(545)**	**209**	**209**	**208**	**(623)**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**356**

3. DISCOUNTED CASHFLOW	**Year 0**	**Year 1**	**Year 2**	**Year 3**	**Year 4**	**Year 5**	**Year 6**	**Year 7**	**Year 8**	**Year 9**	**Year 10**	**Year 11**	**Year 12**	**Year 13**	**Year 14**	**Year 15**	**Year 16**	**Year 17**	**Year 18**	**Year 19**	**Year 20**	**Total 20 Years**
TOTAL PROJECT CASHFLOW £K	**230**	**168**	**181**	**2**	**236**	**211**	**(765)**	**209**	**209**	**208**	**(889)**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
TOTAL CUMULATIVE PROJECT CASHFLOW £K	**230**	**398**	**579**	**581**	**817**	**1,028**	**263**	**472**	**681**	**889**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	

Scottish Macromolecular Imaging Centre
Capacity and Partner Benefit Structure

Purpose

To outline the estimated available capacity and annual running costs for SMIC and the partner benefit structure.

1. Capacity

In the first year, capacity is assumed as follows:

Maintenance/ Down Time =	60 days per year.
Training =	40 days per year.
Holidays =	10 days per year (Christmas shutdown).

Therefore, total available days based on 24/7 running = 255.

Note that Diamond confirmed 257 days of user access delivery in first year of operation of Krios 1.

2. Running Costs

The model is intended to be a basis on which sustainability of SMIC can be maintained. The running costs are assumed to be £407k per annum.

This is comprised of:

Staff Cost (2 FTE)	£108k
Maintenance Contracts	£158k
IT Storage	£68k
Consumables	£63k
Scientific Advisory Costs	£5k (biennial)
Training	£5k (will be £10k every other year)
Total	£407k

The 2 FTE Staff costs are funded from Beatson plus University of Glasgow contributions to SMIC.

The running costs that need to be sustainable recovered by SMIC are therefore **£299k.**

It is expected that the initial purchase of the equipment will include a 5 year maintenance package. The inclusion of maintenance costs in the above figures is designed to ensure a visible build-up of funding to allow renewal of maintenance for 5 years beginning in year 6 and thus give confidence around sustainability.

A similar approach has been taken with IT storage costs.

3. Partner Benefit Structure

In order to put in place a structure that benefits the partners and reflects the funding contributions made from each, the following model is proposed:

a. Gold Subscription:
   - Available to partners who have contributed >£100k to initial capital funding
   - Guarantees availability of 53 days (23 sessions).
   - Each session is charged at a slightly discounted rate based on recoverable cost only (no mark up). This enables the subscriber to accommodate free access to WT funded projects. Based on hourly rates, this is £2,173 per session.
   - Each Gold partner guarantees to pay for all available days. Sub-contracting of days is permissible.

b. Silver Subscription:
   - Available to partners who have contributed >£75k to initial capital funding
   - Guarantees availability of 30 days (13 sessions).
   - Each day is charged at a slightly discounted rate based on recoverable cost. As above, this enables the subscriber to accommodate free access to WT funded projects. Based on hourly rates, this is £2,177 per session.
   - Each Silver partner guarantees to pay for all available days. Sub-contracting of days is permissible.

c. Bronze Subscription
   - This is the mechanism to make SMIC available on a charged basis for the remaining unallocated sessions (28).
   - Each day is charged at cost plus 48% (to reflect the lack of contribution to SMIC infrastructure). Based on hourly rates, this is £5,034 per session.

The table below shows the assumed recovery of costs using this model:

Subscription	Days per Year	Sessions	No of Partners	Days Allocated	Annual Recovery of Cost
Gold	53	23	3	159	£50,000
Silver	30	13	1	30	£28,301
Bronze	66	28	0	66	£140,976
Total Cost Recovery					**£318,976**

Note that there is a potential over-recovery if all 66 "Bronze" days are utilised at £89 per hour. The actual number of days that require to be utilised at that rate for sustainability is 56. This leaves scope for adjustment to rates, days for repeating failed sessions.etc.

SMIC management group would review the use of any unallocated days.

It is expected that SMIC management group would review the model on an annual basis to ensure charges and costs remain appropriate to maintain the sustainability of SMIC.

10. Data management and data sharing

Will the proposed research generate data or software outputs that hold significant value as a resource for the wider research community?	Yes

Please provide a data management and sharing plan

1. Description of the data

1.1 Types of data

The predominant classes of data are:

- Raw grey-scale images and image movies acquired on the transmission electron microscope for single particle imaging
- Tilt-series of grey-scale images acquired on the electron microscope for tomographic analysis
- Computed three-dimensional (3D) image reconstructions from single particle data
- Computed 3D tomograms
- Computed 3D sub-tomogram averages
- Atomic-resolution coordinate files describing macromolecular structures

1.2 Format of the data

Data will be acquired using proprietary software associated with the electron microscope or community developed software (Such as SerialEM - http://bio3d.colorado.edu/SerialEM/)

Electron micrographs – images from the electron microscope for single particle analysis are collected using proprietary software on the instrument.

- For automated collection of both single particle and tomography data images are recorded primarily in MRC format or Gatan's DM4 format

Computed 3D reconstructions will be prepared using a range of community developed software packages including Relion, Frealign, IMOD and Dynamo and stored in MRC format.

2. Data collection / generation

2.1 Methodologies for data collection / generation

Raw data will be collected by electron microscopy. Three-dimensional (3D) reconstructions will be calculated using open-source community developed software.

2.2 Data quality and standards

Data will be collected on a calibrated and manufacturer maintained instrument according to laboratory standardised protocols. Many thousands of images are acquired for 3D reconstruction. Data validation is by accepted methods (Resolution assessed by Fourier Shell Correlation criteria). Ultimately the quality of data will be evaluated in the course of peer-review for publication.

3. Data management, documentation and curation

3.1 Managing, storing and curating data.

We propose to establish a world-class centre for cryogenic transmission electron microscopy with the primary objective of macromolecular structure determination in the life sciences. The facility will comprise an automated cryomicroscope equipped with state-of-the-art direct detection device cameras. Such instruments typically produce 2-3 TB of data per 48-hour session, thus yielding a potential 0.3-0.4 PB of data per year.

To ensure data security SMIC will assume responsibility for storage of primary datasets.

To this end we will purchase a large disk array to receive and store data at close proximity to the facility. To guarantee performance and reliability, management of this system will be contracted to a third-party. For added security, data will be mirrored to local off-site storage and 'live' data backed up at the St. Andrews University Guardbridge data-centre.

Following each data collection session, users will receive their raw data on a USB-hard drive. Management, archive and curation of data generated downstream will be the responsibility of individual investigators but will adhere to accepted community standards, including deposition of published structures and datasets in public databases as set out in 3.3 (below).

3.2 Metadata standards and data documentation

Raw data curation will be managed using EMEN2 (http://blake.bcm.edu/emanwiki/EMEN2). Metadata for raw and processed datasets are captured and recorded either in the header of the image file (tilt-series MRC files, DM4), or in metadata files produced by the image processing software (STAR files - Relion). In the event that an image-processing package does not adequately capture metadata, these will be recorded in electronic laboratory notebooks. Relevant metadata will be deposited in public databases along with the 3D reconstructions generated.

3.3 Data sharing, preservation strategy and standards

Completed datasets, reconstructions and accompanying documentation (manuscripts etc) are archived to magnetic tape for 10 years local storage.

Published reconstructions and coordinates will be deposited in public databases for structural biology; the EMDB and PDB:
http://www.ebi.ac.uk/pdbe/emdb/

http://www.ebi.ac.uk/pdbe/

These will be supported by deposition of raw datasets in public archives with persistent identifiers (i.e. DOI). The primary community resource for cryomicroscopy is EMPIAR – Electron microscopy pilot image archive (http://www.ebi.ac.uk/pdbe/emdb/empiar/). Currently in the early stages of development it is hoped that this will become the reference database for accessing published raw image datasets. In University of Glasgow research data may be archived in the University's *Enlighten* database, where they will be held and publically available for a period of ten years. Institutional repositories will be used in the event that the community project does not serve funders requirements.

11. Contributions

Please list all financial contributions, or equivalent, provided by the host organisation or other sources. Please refer to guidance notes.

Contributor	Details of contribution	Value	Currency
University of Glasgow	Capital contribution to SMIC infrastructure	200,000	Pound Sterling
University of Glasgow	In Kind contribution 1 FTE Laboratory manager 1 FTE Imaging specialist £108,000 per annum Staff total £540,000 Building costs for SMIC microscope room in Sir Michael Stoker Building: 47 m2 (including 7.4 m2 anteroom) occupying 2 floors at overall build cost of £2800/m2 - £263,000 Maintenance contracts for Glasgow spoke facility to support project development. Direct Electron DE20 £22,605 FEI Vitrobot £3,564 JEOL 2200FS £42,745 Total £68,914 per annum = £344,570	1,147,570	Pound Sterling
University of Glasgow	Underwritten subscription access charges £50k per annum	250,000	Pound Sterling
University of Dundee	Capital contribution to SMIC infrastructure	125,000	Pound Sterling
University of Dundee	In Kind contribution 0.5 FTE imaging specialist - to support project development at Dundee spoke facility.	193,018	Pound Sterling
University of Dundee	Underwritten subscription access charges £50k per annum	250,000	Pound Sterling
University of Edinburgh	Capital contribution to SMIC infrastructure	100,000	Pound Sterling
University of Edinburgh	In Kind contribution 0.5 FTE imaging specialist - to support project development at Edinburgh spoke facility. £114,579 Maintenance contract for FEI F20 at Edinburgh spoke facility to support project development. £40k per annum	314,579	Pound Sterling
University of Edinburgh	Underwritten subscription	250,000	Pound Sterling

	access charges £50k per annum		
University of St Andrews	Capital contribution to SMIC infrastructure	75,000	Pound Sterling
University of St Andrews	In Kind contribution 0.5 FTE imaging specialist - to support project development. £150k Access to 500 TB high performance data storage at Guardbridge data centre £200k (estimated) Access to 10 days per annum GPU processing capacity £25k (estimated)	375,000	Pound Sterling
University of St Andrews	Underwritten subscription access charges £28k per annum	140,000	Pound Sterling
Medical Research Council	Capital contribution to SMIC infrastructure	400,000	Pound Sterling
Scottish Funding Council (SFC)	Capital contribution to SMIC infrastructure	100,000	Pound Sterling
Scottish Universities Life Sciences Alliance (SULSA)	Capital contribution to SMIC infrastructure	60,000	Pound Sterling

12. Public engagement

Do you have plans for engaging with the non-academic public about your work?	Yes

Please provide a brief outline of your public engagement plans
Glasgow University's 'Inspiring People' strategy prioritises engagement and Glasgow was ranked as top University in the recent UK-wide BBSRC 'Excellence with Impact' competition, owing to our transformational culture change and engagement with the impact agenda. The University also organises the Glasgow Science Festival (50,000 visitors over 2 weeks) and has a strong strategic partnership with the Glasgow Science Centre (regular exhibits featuring University research and researchers are seen by over 380,000 visitors per annum). David Bhella is a renowned science communicator (winner of the Microbiology Society 2013 Peter Wildy prize - https://youtu.be/X6oVuoNYRAQ) and has a long track record of delivering high-quality schools and public engagement activities. In 2007 David worked with CVR colleagues, artist Murray Robertson and Glasgow Science Centre to produce an art/science exhibition (www.molecularmachines.org.uk) that toured Scotland. In 2014 he organised a microscopy day for primary schools that coincided with the annual Scottish Microscopy Group symposium. Delivered in partnership with JEOL, Thermo-Fisher and the Royal Microscopical Society, pupils enjoyed a morning of hands-on experience with cutting-edge light and electron microscopy. Such events enthuse young audiences showing that high-quality science happens on their doorsteps, making

careers in science both appealing and achievable. Structural biology research produces eye-catching imagery that is intriguing to a lay-audience. Data produced through this project are eminently suitable for use in engagement activities. David will work with network partners and Glasgow's Dean of Public Engagement, Kevin O'Dell to deliver a range of public and schools activities showcasing our world-class structural biology research

Please note that we provide support for Wellcome Trust funded researchers to engage with the non-academic public. Do you wish to receive information about training, funding and other public engagement opportunities?	Yes

13. Location of activity

Will the funded activity take place at more than one location?	Yes

For each location, select the country and, where applicable, state the organisation (please include the administering organisation).	
Country	**Organisation**
United Kingdom	University of Glasgow
United Kingdom	University of Dundee
United Kingdom	University of St Andrews
United Kingdom	University of Edinburgh
United Kingdom	Newcastle University

Will the funds awarded be allocated to more than one location?	No

14. Costs requested and justification

Please select the currency in which you wish to apply.
GBP - Pound Sterling

Is the selected currency your local currency?	Yes

Salaries Are you requesting salaries? Please refer to guidance notes and definition of terms for further details.	No

Materials and consumables Are you requesting materials and consumables?	No

Animals Are you requesting animals?	No

Equipment Are you requesting equipment or equipment maintenance?	Yes

Equipment

Type of equipment	No. of items	Cost per item	Cost of maintenance contract	Contribution from other sources	Total (£)
300 keV automated cryomicroscope with direct detector energy filter and phase plates	1	4294255	0	294255	4,000,000

Access charges Are you requesting access charges?	No

Travel and subsistence Are you requesting travel and subsistence?	No

Miscellaneous costs Are you requesting miscellaneous costs?	No

Justification for resources requested
Please provide a complete justification for all the resources requested, ensuring that you present this information according to the cost headings requested above.

Equipment.

We request funds towards the cost of an automated 300 keV cryomicroscope for single particle and tomographic imaging in the life sciences. To inform our proposal, representative quotes have been obtained from JEOL (**£3,718,990** quotation EOQ-SK-16_035-3) and FEI (**£4,294,255.00** quotation QUO-73001-Q6P4 R0). Included in the purchase cost for both suppliers is an additional four years of service and maintenance. In all our budgetary planning we have listed the higher priced item.

This purchase is on behalf of a consortium of Scottish universities and is intended to create a new centre: the "Scottish Macromolecular Imaging Centre" (SMIC). SMIC will serve the structural and cellular biology research communities of Scotland and Northern England. It will nucleate a network of research interests and bring together the existing cryomicroscopy resources of Scotland to form an integrated 'hub and spoke' capacity.

Three 200 keV FEG cryomicroscopes are in operation in Scotland, a JEOL 2200 in Glasgow, an FEI F20 in Edinburgh and a JEOL 2010F in Dundee. Of these machines, only the Glasgow instrument is equipped with a direct detection camera (DE20). Glasgow and Edinburgh both have limited capacity for automated imaging, but are not suited to unattended operation owing to the side-entry cryostages in use. There are no 300 keV cryomicroscopes in Scotland.

Installation of a 300 keV automated microscope will bring about a step-change in the research capabilities and outputs of the Scottish structural biology community. The improved performance of DDD cameras at 300 keV will yield superior data - capable of delivering structures at better than three angstroms resolution. Automation will greatly increase throughput. Addition of phase plates will yield enhanced contrast, with particular benefits for tomography projects; data recorded at ~0.5

microns underfocus will have an approximate resolution limit of 8 angstroms in subtomogram averaging experiments before CTF correction.

In founding this facility we have sought CVs of researchers that have self-identified as collaborators having projects suited to automated cryo-microscopy. These are listed in section 5 and appended in section 21. Together with the lead and co-applicants we are more than 30 investigators with a strong and immediate interest in this capability. Once established however we foresee further interest from the wider cell biology community as *in-situ* methods become embedded.

Creation of a network of researchers will foster further collaborative opportunities in research and will also lead to the foundation of a strong training network that will support the learning of young researchers entering the field, thereby growing capacity across the UK through the provision of highly-skilled practitioners of this exciting technique.

Summary of financial support requested	
	Total (£)
Salaries / Stipends	0
Materials and consumables	0
Animals	0
Associated animals costs	0
Equipment	4,000,000
Maintenance for existing equipment	0
Access charges	0
Travel and subsistence	0
Miscellaneous other	0
Total	**4,000,000**

15. Full economic costing

Is your organisation based in the UK?	Yes

Is your organisation calculating the full economic cost of this proposal?	No

16. Research involving human participants, human biological material and identifiable data

Does your project involve human participants, human biological material, or identifiable/potentially identifiable data?	No

17. Proposals involving animals

Will the funds on this grant be used directly for animal research?	No

18. Risks of research misuse

Please confirm that you have considered whether your proposed research could generate outcomes that could be misused for harmful purposes.
Confirmed

Have you identified any tangible risks of this type?	No

19. Freedom to operate/conflicts of interest

Describe any freedom to operate issues or potential conflicts of interest that have been identified or that might arise and how these will be or have been addressed.

In particular, please consider the following:

- Do any of the individuals involved in the project hold any consultancies or equities in, or directorships of, companies or other organisations that might have an interest in the results of the proposed activity?
- Will the proposed activity use technology, materials or other inventions that are subject to any patents or other form of intellectual property protection?
- Will any element of the activity be subject to agreements with commercial, academic or other organisations, including arrangements with collaborators named in the grant application, that might lead to intellectual property issues or restrictions?

There are no issues with freedom to operate, conflicts of interest of intellectual property restrictions.

20. Wellcome Trust supported facilities

Will the project be based in one of the following Wellcome Trust supported facilities:

- the Wellcome Trust Sanger Institute
- a Wellcome Trust Centre
- a Major Overseas Programme
- the Francis Crick Institute?

No

21. Appendices

1) Curricula vitae PDF - Combined_CVs_17_10_16.pdf