THE UNIVERSITY OF QUEENSLAND
AUSTRALIA

Institute for Molecular Bioscience
Professor Matthew Cooper, FRSM
NHMRC Principal Research Fellow

CRICOS PROVIDER NUMBER 00025B

30th January 2015

Professor Chris Dowson
Biological Sciences
University of Warwick
Coventry UK

Dear Chris

RE: BACWAN Facility

I write in support for the BACWAN Facility's continued funding. I understand that you are applying for long-term support for the facility and am delighted to provide evidence to emphasise the importance of BACWAN, the assays and reagents it supplies to the antimicrobial research community.

A large class of clinically important antibiotics target peptidoglycan biosynthesis; a key component of bacterial cell walls Advances in our understanding have been held back to a large extent because of the lack of reagents, the biosynthetic intermediates required to study the enzymology of biosynthesis and remodelling.

Development of the synthesis facility at Warwick has now enabled widespread, at cost, access to these key reagents that are not available commercially. We have successfully collaborated with the team at BACWAN on a new class of transgycosylase inhibitors with extremely low resistance induction rates and activity *in vivo.* The availability of the substrates and fluorescently labelled variants of the bacterial substrates involved is real asset to our research and could also facilitate the development of novel drug discovery opportunities, much needed in this era of increasing antibiotic resistance.

I wish you all the best for your upcoming application.

Sincerely,

Professor Matthew Cooper, FRSM
NHMRC Principal Research Fellow

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27th January 2015

Professor Chris Dowson
Life Sciences
University of Warwick
UK

Dear Chris,

Re: BACWAN

I would like to add my wholehearted support to your application to continue the BACWAN Facility. This is a unique set up providing reagents to the bacterial cell wall community, both academia and industry, around the world. The provision of such peptidoglycan biosynthetic intermediates is crucial to our existing and proposed studies to be able to get to grips with how the important pathogen, *Staphylococcus aureus*, is able to grow and divide. The alarming spread of antimicrobial resistance means that understanding the basic mechanisms of peptidoglycan biosynthesis, using the BACWAN reagents, is crucial to the development of new control regimes.

Kind regards,

Professor Simon Foster
Director of the Krebs Institute

DEPARTMENT OF CHEMISTRY

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Christopher J. Schofield FRS
Professor of Chemistry
Head of Organic Chemistry

17 February 2015

Dr David Roper,
School of Life Sciences,
University of Warwick,
Gibbet Hill Road,
Coventry.
CV4 7AL

Dear Dr Roper,

I am very happy to write in support of your application to the Welcome Trust for continued support for the BACWAN peptidoglycan reagent facility. Whilst our group has not yet used BACWAN, with our renewed interest in bacterial cell-wall biosynthetic inhibitors, it is very likely we will do so in the future. I understand that the facility has supported global research in the area of bacterial cell wall biosynthesis in industry and academia. Development of the synthesis facility at Warwick now has enabled access to these key reagents that are not readily available commercially. In my view a facility is extremely important and timely in a period where global efforts to tackle antimicrobial resistance is of paramount importance and the cell wall is a proven target for antimicrobial therapy.

Yours sincerely,

C. J. Schofield

Professor C.J. Schofield, FRS
Head of Organic Chemistry

COLUMBIA UNIVERSITY
College of Physicians and Surgeons

JONATHAN DWORKIN, PH.D.
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29 January 2015

Professor Chris Dowson
Professor of Microbiology
University of Warwick

RE: Support for the BACWAN facility

Dear Dr. Dowson,

I am writing to express my strongest support for the BACWAN facility at the University of Warwick. This facility provides an invaluable service to the international community of researchers working on peptidoglycan through its synthesis and distribution of reagents that are absolutely necessary for our research. There is no other facility like it in the world, and in my experience, it was truly transformative in our research. Specifically, we are interested in the mechanism by which the peptidoglycan precursor Lipid II is flipped across the cytoplasmic membrane before being integrated in the extracellular murein. We have been actively pursuing the mechanistic basis of this translocation using a variety of approaches, some of which are described in recent publications (e.g., *J. Mol. Bio*, 2011; *J. Am Chem. Soc*, 2012) and also some recent unpublished data. In this regard, access to the Lipid I substrate produced by BACWAN has been critical for our progress and I anticipate that additional reagents will be continue to extremely helpful. For example, we are interested in demonstrating cross-linking between Lipid II and a candidate regulator of translation, and for this work, which has recently been funded by the US NIH, Lipid II produced by BACWAN will be essential.

I trust that the Wellcome Trust will see fit to support this important resource.

Best,

Jonathan Dworkin

Laboratoire des Enveloppes Bactériennes et Antibiotiques
CNRS
Bâtiment 430
Université Paris-Sud
91405 Orsay, France

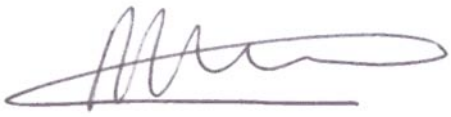
Orsay, February 10th, 2015

To whom it may concern

For more than 40 years, our laboratory « Enveloppes Bactériennes et Antibiotiques » (CNRS) has been studying peptidoglycan metabolism from chemical, biochemical, physiological and genetic point of views. This has in particular led us : i) to decipher the mode of action of, and mechanisms of resistance to, certain antibiotics (vancomycin, bacitracin, colicin M); ii) to elucidate the substrate specificity of peptidoglycan-synthesising enzymes from *Escherichia coli*, and also from important pathogenic bacteria (*Staphylococcus aureus*, *Streptococcus pneumoniae*, *Chlamydia trachomatis*); iii) to assay a considerable number of synthetic compounds as inhibitors of peptidoglycan-synthesising enzymes, and hence as potential antibacterial compounds.

To implement such studies, the availability of peptidoglycan intermediates (UDP-MurNAc-peptides, lipids I and II) in large amounts was a prerequisite. Previously, we prepared these compounds, which were not commercially available, either from natural sources or through chemo-enzymatic synthesis. These procedures were time-consuming and sometimes resulted in low yields. In the recent years, the possibility of getting these intermediates from the BACWAN facility has greatly simplified our work. The interruption of its activity would greatly hamper our research. Therefore, we do hope that the BACWAN facility will continue to provide peptidoglycan intermediates to the community, and we fully support the application that Prof. Dowson and his colleagues are submitting for this purpose.

Yours sincerely



Dr. Didier Blanot
Directeur de Recherche au CNRS

INSTITUTO
DE TECNOLOGIA
QUÍMICA E BIOLÓGICA
/UNL

Knowledge Creation

23th January 2015

I give my full support to the continuation of the BACWAN facility at the University of Warwick.

This facility has been providing the community of researchers studying peptidoglycan metabolism and antibiotic resistance with various extremely useful compounds. Personally, I will require the peptidoglycan precursor lipid II for my studies of resistance against cell wall inhibitors of *Staphylococcus aureus.*

Mariana G. Pinho
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UNIVERSITY of GUELPH

DEPARTMENT OF MOLECULAR AND CELLULAR BIOLOGY

10 February 2015

Professor Chris Dowson
Professor of Microbiology
University of Warwick

Dear Chris

re: Support for the BACWAN facility

I write to express my most strongest support for the BACWAN facility at the University of Warwick. As a totally unique facility in the World that provides a complete array of precursors for peptidoglycan biosynthesis, there is no doubt of its value and service to the international community of researchers investigating peptidoglycan metabolism from both basic and applied perspectives. As you know, our interests concern the understanding of the structure-function relationship of enzymes involved in the modification (specifically O-acetylation) and lysis of peptidoglycan as they represent potential new antibacterial targets. With your help, we aim to polymerize Lipid II into a homopolymer of peptidoglycan repeating units (*viz.* GlcNAc-MurNAc-pentapeptide) which would provide the first consistent substrate for these (and other related) enzymes. Indeed, our studies, and those within the community, have been severely hindered by the lack of a soluble and consistent substrate. However, your ability to provide Lipid II in mg quantities, and your recent production of a homopolymer of uncross-linked peptidoglycan strands, will greatly facilitate our studies. Moreover, I am excited by the opportunity to test if your enzymatic system is able to generate Lipid II derivatives that possess a tetrapeptide, or tripeptide, instead of the natural pentapeptide, and that the transglycosylase would be able to polymerize these metabolites. Such potential additional substrates would help determine the specificity of our enzymes of interest.

I truly hope that the Wellcome Trust will recognize the importance of the BACWAN facility, and commit to supporting its continuance.

With best wishes,

Anthony J. Clarke PhD
Professor & Assoc. Vice-President (Graduate Studies)

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Duke | DUKE UNIVERSITY MEDICAL CENTER
DEPARTMENT OF BIOCHEMISTRY

Seok-Yong Lee, Ph.D.
Assistant Professor of Biochemistry

January 28, 2015

David Roper, Ph.D.
Chris Dowson, Ph.D.

Chris Dowson
Professor of Microbiology
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Dear David and Chris,
I would like to express my strong enthusiasm in supporting your application to the Welcome Trust entitled, "*To develop additional new reagents, make current reagents more efficiently and to enhance our provision of reagents to an expanding research community"*.

My laboratory has been working on structural and mechanistic studies of MraY, an essential enzyme in bacterial cell wall synthesis. MraY is particularly interesting as a potential broad-spectrum antibacterial drug target because there are many different classes of natural product MraY inhibitors (e.g., the capuramycins and muraymycins) that demonstrate potent activity against pathogenic bacteria, such as *M. tuberculosis* and *S. aureus*. Although this validated target has generated a lot of excitement among drug designers, there has been limited progress in developing MraY-targeted antibiotics because MraY is an integral membrane enzyme that has been difficult to isolate for reliable biochemical and structural characterization. Our laboratory made a breakthrough contribution to the field with the first X-ray crystal structure of a member of the MraY family, which was published in ***Science*** (Chung BC et al, 2013).

Part of our success is directly attributed to the work of the BACWAN facility. To characterize the MraY enzyme we expressed and purified, we have used radiolabelled substrates (UDP-MurNAc-pentapeptide) and product (Lipid I) produced by the BACWAN facility since 2012. Without the support of the BACWAN facility, it would have not been possible for scientists like me to enter into the field of peptidoglycan research because the synthesis of radiolabelled peptidoglycan precursors is challenging. I would like to point out that our dependence on the BACWAN facility has further increased with time; we are now working on detailed characterization of our enzyme along with structural studies of this enzyme in complex with substrates and product.

I strongly believe that the BACWAN facility is an essential component in the field of peptidoglycan research, which is gaining more and more attention from both academia and industry. Your facility will significantly contribute to the work conducted in my laboratory and others in the field. I look forward to helping you in any way that I can, and I wish you the best in submission of your application.

Best Regards,

INSTITUTO
DE TECNOLOGIA
QUÍMICA E BIOLÓGICA
/UNL

Knowledge Creation

Oeiras, February 7th 2015

Prof. Chris Dowson
Professor of Microbiology
Biological Sciences
University of Warwick, Coventry , UK

Dear Professor Dowson,

I have learnt that the BACWAN facility, at the University of Warwick, is preparing an application to the Welcome Trust, so that it can continue providing the tools, products and expertise that several research groups, located in UK and abroad, use in their research lines.

I am hereby stating my full and unconditional support to your application. I sincerely hope that your application is successful, as it will allow me to acquire different peptidoglycan components that we use in our research initiatives. We are counting with the help of BACWAN to determine ways to prevent bacteria from concealing their peptidoglycan from an infected host and in this way to find alternative strategies to control bacterial infections.

Yours sincerely,

Sérgio Raposo Filipe

Principal Investigator and research group leader,
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Faculté des Sciences
Centre d'Ingénierie des Protéines
Dr. Mohammed Terrak
Chercheur qualifié F.R.S_FNRS

6 February 2015

RE: Support for the BACWAN facility

Dear Dr. Dowson,

I am writing this letter in support of Dr. Dawson and his colleagues at the University of Warwick and their great effort in maintaining the BACWAN facility which is providing the cell wall research community with the precious peptidoglycan precursors.
I am studying peptidoglycan glycosyltransferases, a validated antibiotic target, and my work relay entirely on the availability of lipid II substrate.

Best regards,

Mohammed Terrak

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HARVARD MEDICAL SCHOOL Department of Microbiology and Immunobiology

Thomas G. Bernhardt, Ph.D.
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February 10, 2015

Professor Chris Dowson
Biological Sciences
University of Warwick
Coventry, UK

RE: BACWAN Facility

Dear Chris,

I write to enthusiastically support the application of the BACWAN facility for long term funding.

The BACWAN facility has developed synthesis methods to make a wide variety of precursor molecules available to the international research community studying bacterial cell wall biogenesis. These precursors are not available commercially and very challenging for individual laboratories to make. The limited availability of these precursors is one of the primary reasons so much remains to be learned about how bacteria build their outer cell wall layer. Advances in this area are sorely needed as we are currently faced with the growing problem of antibiotic resistant bacterial infections. Many of our best antibiotic therapies target the cell wall biogenesis machinery. Thus, a great understanding of the process holds the promise of identifying new weaknesses in the pathway to target with next generation antibiotics.

My laboratory studies the regulation of bacterial cell wall synthases that use the lipid-linked cell wall precursors provided by BACWAN. I am very interested in the facilities plans to extend the range and quantities of reagents available as it will allow us to expand our studies to understand substrate preferences for the synthases and evaluate the effect of regulators and inhibitors on their activity.

In closing, I give my full support to the continuing efforts of BACWAN and hope that you are successful in the upcoming application. The community will truly benefit from your success.

Sincerely,

Thomas G. Bernhardt, Ph.D.

university of groningen / faculty of mathematics and natural sciences / molecular microbiology

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Prof. Christopher Dowson
Life Sciences
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University of Warwick
Coventry
CV4 7AL, UK

Date
26 January 2015

Our reference

Subject
Bacwan support letter

Dear Professor Dowson,

I am writing this letter in support of the Bacwan facility, that is supplying the peptidoglycan research community with much needed reagents. Peptidoglycan is the most conserved bacterial molecule and the target for many antibacterial compounds such as penicillin (derivatives), but also antibacterial peptides and the newly discovered, highly exciting, teixobactin molecule (*1*). The rise of antimicrobial resistance is a great concern for healthcare as acknowledged by governments worldwide, including the UK and the EU. Peptidoglycan, and especially it's building block, LipidII, are considered an Achilles' heel to target multi-drug resistant bacteria (*2*).

Work on Peptidoglycan synthesis requires access to the complex chemicals that make up Peptidoglycan. My lab is currently moving from *in vivo* work that addresses questions on the location of Peptidoglycan synthesis (*3*) to more defined systems to address questions related to the mode of action of antibacterial peptides that target LipidII (under review), and the recycling of Peptidoglycan fragments. For this work we will most certainly need access to such fragments, which are routinely synthesized and made available by the BAcwan facility. Ordering these compounds from other suppliers is impossible – they are simply unavailable – and making these reagents ourselves requires an enormous investment of both time and money. The services provided by the Bacwan facility are of great importance for the community researching new ways to block bacterial cell wall synthesis, including my group. I therefore wholeheartedly support your application to the Wellcome Trust "To develop additional new reagents, make current reagents more efficiently and to enhance our provision of reagents to an expanding research community".

I hope the Wellcome Trust will fund the continuation of this important service.

Kind regards,

Dirk-Jan Scheffers, Ph.D.
Assistant Professor, Molecular Microbiology, University of Groningen

1. L. L. Ling *et al.*, *Nature* **517**, 455-459 (2015).
2. T. Schneider, H. G. Sahl, *IJMM* **300**, 161-169 (2010).
3. M. C. Lages, K. Beilharz, D. Morales Angeles, J. W. Veening, D. J. Scheffers, *Environmental Microbiology* **15**, 3272-3281 (2013).

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universitätsklinikumbonn

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Pharmazeutische Mikrobiologie, Meckenheimer Allee 168, D-53115 Bonn

Institut für Pharmazeutische Mikrobiologie

Prof. Dr. Tanja Schneider

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DZIF Deutsches Zentrum für Infektionsforschung

TTU Novel Antiinfectives

Prof. Chris Dowson
Life Sciences
Room C149.2
University of Warwick
Coventry
CV4 7AL, UK

Bonn, 18. February 2015

Letter of support for the BACWAN facility at the University of Warwick

Dear Prof Dowson,

It is my pleasure to write in support of the continuation of the BACWAN facility at the University of Warwick. The BACWAN facility is a most important source for hard to purify cell wall precursors, especially the lipid-bound peptidoglycan intermediates, which are not commercially available.

Apart from the importance in studying the enzymatic reactions and processes involved in building the bacterial cell wall, these reactions represent most relevant targets for antibiotic intervention.

It is therefore crucial for the scientific community to have access to these high quality reagents that BACWAN can provide. We have been using selected purified precursors in the past (i.e. Ma *et. al*, JBC, 2011). Limiting the access to these most important reagents will be a major drawback for both, basic science and antibiotic discovery.

Prof. Dr. Tanja Schneider

INRA
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Etablissement public à caractère scientifique et technologique
placé sous la tutelle conjointe des ministres chargés de la recherche et de l'agriculture

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Letter of support for the BACWAN facility at the University of Warwick

Jouy-en-Josas, February, 19th, 2015.

With this letter, I want to express my strongest support for the BACWAN facility at the University of Warwick. In the past years, we have used Bacwan facility to obtain a modified peptidoglycan precursor that allowed us to study vancomycin resistance in *Clostridium difficile* (Peltier et al., 2013, Microbiology). We have been involved for many years in the study of peptidoglycan metabolism and in particular in the study of peptidoglycan modifying enzymes that could represent new targets for antibacterial molecules. In our future projects, we will need putative substrates for these enzymes such as lipid II or peptidoglycan precursors that we can obtain only from Bacwan.

Marie-Pierre Chapot-Chartier
Research Director at INRA
Micalis Institute
France

Institut de Biologie et Chimie des Protéines
Bases Moléculaires et Structurales des Systèmes Infectieux
Unité Mixte de Recherche n° 5086 - CNRS / Université Lyon 1

Christophe Grangeasse, PhD, Research Director, CNRS.
Group leader « Bacterial Pathogens and Protein Phosphorylation »
Group website http://perso.ibcp.fr/christophe.grangeasse/
Tel : +33 4 72 72 26 88
Email : c.grangeasse@ibcp.fr

Lyon, February 7th 2015

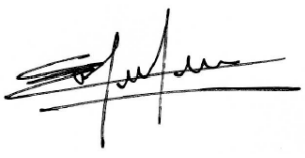
I fully support the application of BACWAN facility at the University of Warwick to the Welcome trust "To develop additional new reagents, make current reagents more efficiently and to enhance our provision of reagents to an expanding research community".

My work focuses on the role of protein phosphorylation in cell division and morphogenesis of *Streptococcus pneumoniae*. Several reports have shown that protein phosphorylation influences the synthesis, the export and the assembly of peptidoglycan, which is the polymer maintaining the bacterial cell shape and the target of many antibiotics. For my work, I will require access to peptidoglycan precursors that are unique to BACWAN to determine how protein phosphorylation regulates and contributes to the assembly of the peptidoglycan polymer.

Despite efficient therapies, antimicrobial resistance among pneumococci has escalated and incidence of infections caused by non-vaccine serotypes has increased over the past decade. Pneumococcal septicemia is a major cause of infant mortality in developing countries, where it causes more than 1.2 million infant death per year. Therefore, there is an urgent need for new antibiotics. In this context, studying peptidoglycan biosynthesis should pave the way for the identification of potential antibacterial targets and design of new molecules for optimal prevention of pneumococcal infections.

I hope that BACWAN will be able to keep on providing researchers with peptidoglycan precursors and that BACWAN will benefit from the support of the Welcome trust.

Dr. Christophe Grangeasse



INDIANA UNIVERSITY
MOLECULAR AND CELLULAR
BIOCHEMISTRY DEPARTMENT
College of Arts and Sciences
Bloomington

February 13, 2015

David Roper
Adrian Lloyd
Chris Dowson
BACWAN Facility
University of Warwick
Warwick, England

Greetings,

It has been brought to my attention that the BaCWAN facility at the University of Warwick is in danger of closing. After almost a decade of support, the funding from the UK Medical Research Council is scheduled to end this December, 2015 and the facility will be forced to terminate its activities. This would be a tragedy because the peptidoglycan intermediates that are made available by your scientists are not readily available from other sources. These reagents are critical for basic research studies of cell wall metabolism.

Antibiotic resistance has become frontpage news, and the need for new agents is critical. Some research groups, including my own, are quite concerned about the problem and are working to discover and test new antibacterial agents. Our experience over the history of antibiotic research is that agents that affect the synthesis of the bacterial cell wall are some of the most successful drugs in the history of the pharmaceutical industry. However, new agents that act on the bacterial cell wall are not always easy to characterize. Validation of novel mechanisms of action for new antibiotics depends on having a reliable source for difficult-to-synthesize peptidoglycan intermediates. BaCWAN has been serving that purpose for the past decade. I hope that the Wellcome Trust will consider providing funding for this valuable and critical function.

Sincerely,

K Bush

Karen Bush, PhD
Professor of Practice in Biotechnology
Adjunct Professor of Biology and of Molecular & Cellular Biochemistry

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From: Dr Mark G. Moloney
Professor of Chemistry and EP Abraham Fellow in Chemistry, St Peter's College

March 3, 2015

Dr David I Roper

Reader in Structural Biology

School of Life Sciences,

University of Warwick

Gibbet Hill Road,

Coventry,

CV4 7AL.

Dear David,

Continuation support for the BACWAN facility

It is my pleasure to write to you to support your application for continuation funding for the BACWAN facility at Warwick University. As an academic synthetic chemist and a relative newcomer to antibacterial research, we have spent the last decade developing viable chemistry for the rapid and effective preparation of compound libraries with antibacterial activity. Our initial approach has been to identify library members with antibacterial phenotypic activity, followed by a second phase deconvolution to establish mode of action. Preliminary data suggests that our compounds are cell wall inhibitors with unusual modes of action. We are now moving to more detailed characterisation in this second phase, and in this regard the BACWAN facility will provide an essential underpinning service, providing reagents which would otherwise not be available.

Given the re-emergence of antibacterial research as a national priority of substantial urgency, BACWAN should be regarded as a key national infrastructural asset whose continuation is essential. I fully support this application.

Yours Sincerely,

Mark McLoney

Institut de Biologie Structurale Jean-Pierre Ebel

Groupe Pneumocoque

12th February 2015

Re : Support BACWAN facility

Dear Pr Dowson,

We are writing to express our strong support for the BACWAN facility at the University of Warwick. This facility provides crucial reagents to the international community of scientists studying bacterial cell wall. This service is currently unique in the world.

In our time of worldwide bacterial resistance to antibiotics, research in the field of bacterial cell wall is strong and expanding, and largely relies on tools offered by the BACWAN facility.

Our lab has reported the first in vitro synthesis of peptidoglycan from a Gram-positive bacteria, using the precursor lipid II from BACWAN (Zapun et al. 2013 ACS Chem Biol). This breakthrough (after 40 years of failed attempts) opens the way to the study of penicillin resistance in the pneumococcus. We have recently obtained two three-years grants from French agencies to pursue this project, including sums ear-marked to purchase essential reagents from BACWAN.

We sincerely hope that the Wellcome Trust will continue to support BACWAN for the years to come.

Regards,

Dr. Thierry Vernet
Institut de Biologie Structurale
Grenoble, France

Dr André Zapun
Institut de Biologie Structurale
Grenoble, France

Clare Bryant
Professor of Innate Immunity,
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The University of Cambridge,
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Fellow of Queens' College
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UNIVERSITY OF CAMBRIDGE

Department of Veterinary Medicine

Tuesday, February 10, 2015

Letter of support for the BACWAN facility at the University of Warwick

Dear David and Chris,

I am writing in support of your application for further funding of the BACWAN facility. My research group has a long-standing interest in the study of how peptidoglycan interacts with cells and its functional significance in host-bacterial interactions. This work requires access to high specific peptidoglycan structures made available through the BACWAN facility in order to be able to accurately interpret the precise molecular mechanisms underlying host cell activation.

BACWAN therefore represents a critical resource for my research and all related applications aimed at understanding how bacteria interact with host immune cells. I am enthusiastically supportive of the BACWAN facility.

Yours sincerely

Clare E. Bryant

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January 23, 2015

Professor Chris Dowson
Biological Sciences
University of Warwick
Coventry UK

RE: BACWAN Facility

Dear Chris,

It is a real pleasure to write a strong letter of support for the BACWAN Facility. I understand that you are applying for long-term support for the facility.

I am delighted to confirm that the Warwick group have developed a synthesis facility that is now of real international importance. Peptidoglycan is a key component of bacterial cell walls yet there are still fundamental gaps in our knowledge of the pathway involved in its synthesis, polymerisation and remodelling. Advances in our understanding have been held back to a large extent because of the lack of reagents, the biosynthetic intermediates required to study the enzymology of biosynthesis and remodelling.

Development of the synthesis facility at Warwick has now enabled widespread, at cost, access to these key reagents that are not available commercially. Furthermore the current plans to extend the range and quantities of reagents will open up new areas of research. While we have not yet used the facility I am very interested in intermediates in the difficult late stages of the pathway, for example, lipid I and lipid II. The availability of the substrates and fluorescently labelled variants would be a real asset to my own work in wall teichoic acid biosynthesis and could also facilitate the development of novel drug discovery opportunities, much needed in this era of increasing antibiotic resistance.

Again, this facility and its leadership have my highest regard and support. I wish you all the best your upcoming application.

Sincerely,

Eric Brown, Ph.D.
Canada Research Chair in Microbial Chemical Biology

Imperial College London
100 years of living science

Section of Microbiology & MRC Centre
for Molecular Bacteriology and Infection
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Angelika Gründling Ph.D.
Reader in Molecular Microbiology

14th February 2015

Professor Christopher Dowson
Professor of Microbiology
University of Warwick

RE: Support for the BACWAN Facility

Dear Dr. Dowson,

I am writing this letter to express my strong support for the application of the BACWAN facility at the University of Warwick to obtain grant support for a continuation of their activities and the production of high quality and purity peptidoglycan synthesis intermediates, which are otherwise not commercially available.

As part of my Wellcome Trust New Investigator Award, we are investigating the function of the essential signaling nucleotide c-di-AMP in *Staphylococcus aureus*. Interestingly, we and now also other research groups have found that an increase in the cellular levels of this nucleotide leads to increased methicillin resistance in *S. aureus*. However the mechanistic details are currently not known. Our work has shown that this increased methicillin resistance correlates with changes in the overall peptidoglycan structure (*PLOS PATHOGENS*, 2011, Vol: 7, ISSN: 1553-73740). To determine specifically, which step in the peptidoglycan synthesis pathway is controlled by the c-di-AMP signaling nucleotide and results in increased methicillin resistance, we are in the process of performing a detailed analysis of the different peptidoglycan intermediates. For this work, it will be essential for us to be able to obtain in the future different peptidoglycan synthesis intermediates as controls and internal standards. These are reagents, which are currently produced by the BACWAN facility and could not obtain from other sources. Therefore, I very much hope that funds will be available for the BACWAN facility to be able to provide these reagents also in the future, which are invaluable to my own research and to the peptidoglycan research community as a whole.

Yours sincerely,

Angelika Gründling

Professor Richard J. Lewis, MA, D.Phil

Institute for Cell and Molecular Biosciences
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Professor Chris Dowson
School of Life Sciences
Gibbet Hill Road
University of Warwick
Coventry, CV4 7AL, UK

27th January 2015

Dear Chris

Re: The BACWAN facility

I write in full support of your application to the Wellcome Trust to continue the support of the BACWAN facility in Warwick. BACWAN can synthesise many of the precursors in the synthesis of peptidoglycan, the essential polymer that surrounds all bacteria, and which is the target of mankind's most successful antibiotics. In an era when antimicrobial resistance has become prevalent, and is the focus of the 2015 Longitude prize, it is imperative that genuine progress is made in our understanding of antimicrobial resistance and the mechanisms employed by bacteria to synthesise, polymerise and re-model peptidoglycan. It is also essential to understand how these phenomena are linked to cell division. However, the study of peptidoglycan biochemistry has been hampered across the world because of the unavailability of key peptidoglycan intermediates. The advances, however, made in recent years by BACWAN have ensured that some of these derivatives have become available for the first time. Lipid II, for instance, is of especial interest to my group as we seek to understand how peptidoglycan is polymerised by penicillin binding proteins (PBPs), and how this process is controlled by PBP regulators that are involved in the co-ordination of cytokinesis. For instance, my lab is currently focussing on the effect of essential cell division regulators EzrA and GpsB on the enzymatic activity of the major cell division PBP, PBP1A. We are also mapping these interactions biochemically and structurally, and a major future goal is the structure determination of PBP1A in complex with a PG fragment, such as lipid II, with and without these cell division regulators and others. To achieve success, we are dependent upon others for the supply of PG intermediates, and here the BACWAN facility is going to be essential for us in these endeavours.

Furthermore, and as I'm sure you're aware, my group works closely with that of my colleague, Waldemar Vollmer, a current Wellcome Trust Senior Investigator. In the last year alone, we have co-published structural analyses of the PBP activator, LpoB (Jean et al *Structure* **22**, 1047-1054), the PBP1A regulator, EzrA (Cleverley et al *Nature Commun* **5**, 5421) and the PG LD-carboxypeptidase, LdcB (Hoyland et al *Structure* **22**, 949-960), where all the structural analyses that were driven by my group were complemented by functional studies driven by Waldemar. I envisage that we will retain this close and very successful working relationship in the future, which will depend upon access to compounds that are unique to BACWAN, underpinned by joint applications to funding agencies in the area of antimicrobial resistance and the synthesis of the bacterial cell wall.

Wishing you every success in your application

Yours sincerely,

Prof R J Lewis

CUBIST

February 17, 2015

Professor Chris Dowson
University of Warwick
C.G.Dowson@warwick.ac.uk

Dear Dr. Dowson,

I am writing to give strong support for the BACWAN facility at the University of Warwick.

Peptidoglycan biosynthesis is one of the most important targets for antibiotic therapies. Lack of readily available peptidoglycan reagents has hampered the development of new agents. Through the production of peptidoglycan reagents and the development of new assays enabled by these specialized reagents, antibiotic drug discovery by our company as well as other companies throughout the world is facilitated. This is urgently needed to address the problem of antibiotic resistant pathogens.

Cubist Pharmaceuticals has had many interactions with the BACWAN staff through which we have obtained high quality Lipid II reagents. Of critical importance to us, the speed and efficiency of purchases were vital towards the initiation and execution of a drug discovery project. Future use of the BACWAN facility involving the research proposal on "Tools and reagents for next generation inhibitor discovery in peptidoglycan biosynthesis" is anticipated.

I hope the Wellcome Trust will continue to support the important services provided by the BACWAN facility.

Sincerely,

Aileen Rubio, Ph.D.
Director, Infectious Diseases
Cubist Pharmaceuticals, Inc.

65 Hayden Avenue, Lexington, MA 02421 P 781.860.8660 F 781.861.0566 www.cubist.com

The University Of Sheffield.

DEPARTMENT OF MOLECULAR BIOLOGY & BIOTECHNOLOGY

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UK
Tel: 00 44 1142 224 405
E-mail: s.mesnage@sheffield.ac.uk

26th January 2015

Letter of support for the BACWAN facility at the University of Warwick

Dear David,

It is a pleasure to write a letter in support of the BACWAN facility. As you know, I have a long-standing interest in the study of peptidoglycan metabolism. The work carried out in my laboratory is focused on the analysis of catalytic properties of peptidoglycan hydrolases and the mechanisms underpinning protein-peptidoglycan interactions. Both topics are strongly dependent on the use of peptidoglycan fragments that are not commercially available, such as most of those accessible through the BACWAN facility.
BACWAN therefore represents a critical resource for my research and all related applications aimed at designing novel antimicrobial strategies. As discussed recently, my project is particularly dependent on the availability of uncrossed-linked peptidoglycan fragments with defined glycan chain length. Such fragments are extremely difficult to isolate, but I am confident, based on our discussions that you have the capacity to develop their production at a mg scale.

I am enthusiastically supportive of the BACWAN facility. Peptidoglycan has remained a target of choice for the development of novel antimicrobials since the discovery of penicillin. It is crucial for the scientific community to have access to the reagents that BACWAN can provide.

Yours,

Stéphane Mesnage

Changjiang Dong, Ph.D
Professor of molecular medicine
Wellcome Trust investigator
Norwich Medicine School
University of East Anglia
Norwich Research Park
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UK
Email: C.Dong@uea.ac.uk
Tel:0044-1603-591739

Wellcome trust Biomedical Resources committee

Febuary 17, 2015

RE: Letter of support

Dear WT committee,

I would like to express my support for the application of BACWAN facility at Warwick as a Biomedical Resource for the research community. Bacterial peptidoglycan is an essential component and is a novel drug target for antibiotics. However, the details of molecular mechanisms of the peptidoglycan biosynthesis and assembly are not very clear, due to lacking tools and reagents for the studies. The BACWAN facility has provided the important reagents and tools for the studies of the bacterial peptidoglycan, which I may use for my future work in the bacterial cell wall assembly.

Therefore, I support this application!

Sincerely yours,

Changjiang Dong

February 10th 2015

Dr Roger C. Levesque, Director
IBIS Institute, Laval University
Charles-Eugene Marchand Bld.
1030 Medicine Avenue
Québec City Canada
E-mail: rclevesq@ibis.ulaval.ca.

Letter of support for the BACWAN facility at the University of Warwick

Dear David and Chris,

It is a pleasure and a privilege to write a letter of support for the BACWAN facility. My laboratory was one of the first to publish on *in vitro* synthesis of the peptidoglycan moiety and its enzymatic characterization and subtrate specificity. This could never have been possible without the BACWAN facility where the facility have been producing reagents for this pathway not only for my laboratory but for dozens of laboratories around the world, for biotechnology and for pharmaceuticals.

The peptidoglycan pathway represents one of the key antibacterial targets in bacteria. For many years scientific research in this area was limited by the difficult if not impossible access to the reagents and substrates involved. Even today these reagents are not commercially available. The ease and facility by which BACWAN has made reagents available has created a major regain of interests in peptidoglycan biosynthesis and its great potential for identification of novel antibacterial agents and their analysis. For example, the last four years have seen a major explosion in laboratories now doing peptidoglycan research. This is clearly reflected by the hundreds of scientists from around the world now participating in the “Great Wall Meetings” organized by the BACWAN team. Another example is the number of high impact publications appearing in the scientific literature and the hope for novel antibacterials desperately needed.

My own laboratory has benefited immensely not on only in terms of reagents and publications with members of BACWAN but also in terms of major financial support from the Canadian Institute for Health Research obtaining a CIHR-UK team grant.

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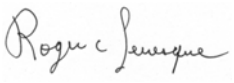
IBIS

An important part of my research absolutely depends on the BACWAN facility and has lead to the development of antimicrobial peptides and small molecules of high therapeutic value.

I am convinced that the BACWAN is a facility of world expertise and excellence that warrants to be supported. This facility is critical to the development of novel antimicrobials that are critically needed in medicine. It is crucial that the world scientific community has access not only to the reagents but also to the unique expertise of BACWAN members.

Sincerely,



Dr. Roger C. Levesque

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COMENIUS UNIVERSITY IN BRATISLAVA
FACULTY OF NATURAL SCIENCES
Department of Biochemistry
Mlynská dolina CH-1, 842 15 Bratislava 4, Slovakia

Letter of Support

I would like to strongly support the application of BACWAN facility at the University of Warwick to obtain the grant for continuation of their activities in providing the reagents and developing new compounds for peptidoglycan research.

As a researcher working in the field of mycobacterial cell wall biosynthesis, but particularly focusing on its mycolyl-arabino-galactan portion, I started to think about extending our attention to peptidoglycan about a year ago. Obtaining UDP-MurNAc pentapeptide, both cold and radioactive, was critical for our experiments and we were getting ready to try producing them in our laboratory. Based on literature the procedure seemed to be rather demanding and I believe it would have required a lot of optimization on our side. Hence I was indeed relieved after I found out that there is a producer of these specific compounds – the BACWAN facility. I have contacted the facility and was pleased with their fast response, overall communication and services. The products which we recieved [UDP-MurNAc-tripeptide (DAP), radiolabelled and cold UDP-MurNAc-pentapeptide (DAP)], were of high quality, with the attached analytical datasheets. So far we have published one paper, in which these compounds were used (Singh V, Brecik M, Mukherjee R, Evans JC, Svetlikova Z, Blasko J, Surade S, Blackburn J, Warner DF, Mikusova K, Mizrahi V. The complex mechanism of antimycobacterial action of 5-Fluorouracil. Chem Biol, 2015; 22: 63-75). This work was part of a collaborative research within the MM4TB (More Medicines for Tuberculosis) consortium supported by European Community's Seventh Framework Programme (grant 260872). In addition, we have performed a number of other experiments with these reagents and based on our encouraging preliminary data concerning peptidoglycan biosynthesis in mycobacteria we were planning their further use in our projetcs relying on the opportunity to obtain the compounds again from the facility.

Obviously, biosynthesis of bacterial peptidoglycan still presents a very attractive and validated target for the development of new antibiotics. In the Tuberculosis Drug Pipeline (http://www.newtbdrugs.org/pipeline.php) there are currently two compounds in pre-clinical development, which target peptidoglycan biosynthesis (CPZEN-45 and SQ641) and thus availability of the reagents produced by BACWAN can significatly promote the research in this area leading to progress of these molecules to further stages and maybe even to so much needed novel medicines. I very much hope that the facility will be able to continue in their activities and provide invaluable support to peptidoglycan research community.

Bratislava, 5th February, 2015

Yours sincerely,
Katarína Mikušová

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CHRISTOPHER DAVIES, PH.D., PROFESSOR
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Dr. David Roper,
Reader in Structural Biology,
School of Life Sciences
University of Warwick
Coventry
CV4 7AL
U.K.

20 February 2015

Dear David,

I want to convey my strongest support for the continuation of the BaCWAN resource. By setting up a facility that provides key species of peptidoglycan (PG) to the scientific community, you have performed a great service to the many investigators who work in the area of peptidoglycan metabolism across the world. These molecules are difficult to purify or synthesize and to do so is beyond the means of most labs. I am amazed and impressed that you have not only managed to purify numerous PG species at milligram level, but are willing to provide them at reasonable cost to the scientific community. You have my admiration and gratitude for doing so.

The BaCWAN resource is unique and its loss would be a setback for my research and that of many others. Its continuation is even more critical due to the worldwide threat posed by antibiotic-resistant bacteria. As you know, I work on penicillin-binding proteins (PBPs). These are transpeptidases that are the lethal targets for β-lactam antibiotics, one of the most significant classes of antibiotic used clinically since the 1940s. Unfortunately, the value of PBPs as clinical targets is being eroded by resistance mutations that render them less susceptible to β-lactams. To address this, we need new generations of PBP inhibitors. One approach is to design inhibitors based on the muropeptide substrate. We cannot do this, however, unless we have a molecular understanding of the interactions made by PBPs with their PG substrate. Historically, this has been a problem because it has been impossible to obtain PG species in sufficient quantities. The BaCWAN facility, therefore, serves a vital function to enable these types of investigation. We are beginning a major new investigation of how *N. gonorrhoeae* PBP2 interacts with PG in order to understand how resistance mutations can discriminate against β-lactams without loss of essential transpeptidase function. This goes to the heart of how bacteria achieve antibiotic

resistance and we anticipate it will lead to new approaches to develop PBP inhibitors. So I cannot overstate the value that your facility brings to my research and I know the same is true for many others.

Finally, let me say that the very existence of BaCWAN brings kudos to the University of Warwick and to the Wellcome Foundation who supports it. It is a unique resource and it is a vital component in our fight against antibiotic-resistant bacteria.

Sincerely yours,

Christopher Davies, Ph.D.

University of St Andrews

James H. Naismith FRS FRSE FMedSci

Bishop Wardlaw Professor of Chemical Biology
Royal Society Wolfson Merit Award Holder
Director of the Biomedical Sciences Research Complex

Tuesday, March 3, 2015

David Roper
University of Warwick
BACWAN Peptidoglycan reagent facility

Dear David,

I am writing to confirm my enthusiastic support for your facility. My lab works on the class of enzymes that synthesis and transport lipid linked sugars through the bacterial cell. The work is funded by the Wellcome Trust. As you know these reagents are like gold dust, we ourselves were unable to source just such a compound for several months last year, disrupting our work. Often the intermediates are not stable for prolonged periods so stockpiling is not a good option.

I would imagine we will be one of your earliest collaborators if you can deliver this facility.

Yours sincerely,

Professor James Naismith

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e-mail: naismith@st-andrews.ac.uk http://chemistry.st-andrews.ac.uk/staff/jhn/group/

Department of Biochemistry and Molecular Biology
Life Sciences Centre
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THE UNIVERSITY OF BRITISH COLUMBIA

Feb. 24, 2015
Professor Chris Dowson
Biological Sciences
University of Warwick
Coventry UK
Re: Support of BACWAN

Dear Chris,

I am writing to provide my strongest endorsement of your application to the Wellcome Trust for funds to support the BACWAN Facility you and your colleagues have created at U. Warwick. BACWAN represents a singly unique, globally available source of the highly complex, and essential substrates to probe perhaps the greatest drug target in history, the cell-wall biosynthetic pathway of bacterial pathogens. BACWAN is and should continue to be the major driving force in providing these reagents that are largely beyond the synthetic scope of the majority of academic and biotechnology laboratories worldwide.

The particular emphasis of my research is the atomic level analysis of several of the membrane-anchored enzymes involved in cell-wall peptidoglycan and teichoic acid synthesis by x-ray crystallographic approaches. This technique is notorious for requiring relatively large amounts of bound substrates for co-crystallization studies with the cognate enzyme targets, complexes essential to understanding the detailed catalytic mechanism and subsequently structure-guided inhibition for drug discovery. BACWAN has been able to provide these substrates at the needed yield and purity, paving the way for eg. of our understanding of lipid II (substrate) binding to the PBP2 enzyme of the notoriously antibiotic resistant Methicillin Resistant *Staph. aureus* pathogen.

I wish you all success in procuring funding for this essential facility on the behalf of the cell-wall community.

All the best,

Natalie C.J. Strynadka, PhD, FRSC
UBC Distinguished Professor of Biochemistry
CRC Tier I in Antibiotic Discovery
HHMI Senior International Scholar

University of Wisconsin
SCHOOL OF MEDICINE
AND PUBLIC HEALTH

February 6, 2015

Professor Chris Dowson
University of Warwick
C.G.Dowson@warwick.ac.uk

Dear Dr. Dowson,

I am writing in strong support of the BACWAN facility at the University of Warwick. This facility is the only place we can purchase certain synthesized peptidoglycan fragments necessary for our studies. We have used UDP-MurNAc-pentapeptides for our studies of peptidoglycan-degrading enzymes in the bacterial pathogen *Neisseria gonorrhoeae*, and we will need more of these molecules and related compounds for our future studies. Peptidoglycan fragments released by *N. gonorrhoeae* cause the tissue damage of gonococcal pelvic inflammatory disease in women. Compounds from the BACWAN facility are being used in my lab in our studies of how the toxic peptidoglycan fragments are made, which ones trigger the pathology, and how the peptidoglycan-degrading enzymes may be inhibited. Publications describing our work using these compounds include: Stohl et al. 2012. J. Biol. Chem 287:11222. and Dillard. 2014. Peptidoglycan metabolism and fragment release. *In* Pathogenic Neisseria: Genomics Molecular Biology, and Disease Intervention. Davies & Kahler, Eds.

Antibiotic resistance in *N. gonorrhoeae* was recently named one of three urgent threats to public health in the United States. We and others are seeking new targets for antimicrobial therapy, and we are evaluating peptidoglycan degradation as a target. Studies such as these will require enzymatic analyses that utilize pure peptidoglycan fragments such as those provided by the BACWAN facility. We look forward to the continuation of the facility and its provision of these necessary and unique compounds.

Sincerely,

Joseph P. Dillard, Ph.D.
Associate Professor of Medical Microbiology and Immunology
University of Wisconsin-Madison
(608) 265-2837 jpdillard@wisc.edu

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Meckenheimer Allee 168
53115 Bonn
Deutschland

Prof. Chris Dowson
Life Sciences
Room C149.2
University of Warwick
Coventry
CV4 7AL, UK

Bonn, 17. February 2015

Letter of support

Dear Professor Dowson,

It is my great pleasure to write a letter of support for continuation of the worldwide unique BACWAN facility. BACWAN is supplying the community of researchers working on peptidogylcan with essential cell wall precursors that are more than difficult to synthesize and not available commercially.
In my junior research group we are currently using and will further depend on BACWAN reagents for research on the cell wall biosynthesis machinery in cell wall envelope lacking *Chlamydiaceae*.

Sincerely,

Beate Henrichfreise, Ph.D.

THE UNIVERSITY OF
WARWICK

13th February 2015

Dear Professor Dowson, Dr Roper and Dr Adrian Lloyd

Re. Support for the BACWAN facility

I am writing to express my support for your application to the Wellcome Trust for funding of the BACWAN peptidoglycan reagent facility at the School of Life Sciences, University of Warwick. I believe that this resource is crucial to be able to provide key peptidoglycan intermediates to the international community at affordable prices. Without such provision of these key synthetic molecules, many of which are not commercially available and are involved in bacterial cell wall biosynthesis, - major bottlenecks in this important area of research would occur. This would be particularly untimely should research halt in this area, given the current global efforts in tackling antimicrobial resistance and for the development of new antibiotics – of which the cell wall/peptidoglycan biosynthesis represents an important target.

I am currently a Sir Henry Dale funded by the Wellcome Trust and The Royal Society. I am interested in nutrient uptake and metabolism in *Mycobacterium tuberculosis*. I envisage that as part of my future research the reagents currently provided, along with any future reagents, by the BACWAN facility will be required in order to enable me to track metabolism and incorporation of these essential biomolecules into the cell wall of the pathogenic organism *M. tuberculosis*.

I hope that the Wellcome Trust will support this resource for the global community.

Yours sincerely

Dr Elizabeth Fullam

School of Life Sciences

The University of Warwick
Coventry CV4 7AL United Kingdom
Tel: 02476 574239

Email: e.fullam@warwick.ac.uk

-

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HEAD OF DIVISION: PROF. DR. KAMIL ÖNDER

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Regarding: BACWAN SUPPORT

Salzburg, 10.2.2015

Peptidoglycan research is more than challenging, especially invitro enzyme reactions are not possible without lipid I and lipid II precursors. To our knowledge there is no other resource existing able to deliver such precursors to scientists. Without BACWAN we would really come into troubles to do our daily research, and it would be a pity for many researches worldwide.

Therefore BACWAN must be fully supported in order to provide researchers with these crucial and hard to produce reagents.

With kind regards,

Prof. Dr. Kamil **Önder**,
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Department of Dermatology,
General Hospital Salzburg
Paracelsus Medical University Salzburg
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Institute for Cell and Molecular Biosciences
University of Newcastle upon Tyne
Faculty of Medical Sciences
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NE2 4HH

Professor Chris Dowson
School of Life Sciences
Gibbet Hill Road
University of Warwick
Coventry, CV4 7AL, UK

Date: 12th February 2015

Supporting Letter BACWAN Facility

Dear Chris,

I am writing to express my strong support for your application to the Wellcome Trust entitled "*To develop additional new reagents, make current reagents more efficiently and to enhance our provision of reagents to an expanding research community*".

As you know my group works on the structure and biosynthesis of bacterial cell wall peptidoglycan in a range of different species. One of our main aims is to decipher the molecular mechanisms of peptidoglycan growth. We have been working mainly in the Gram-negative model bacterium *Escherichia coli*, and now expand our research on other species, where we study the activities and interactions of essential cell wall enzymes, including Penicillin-binding proteins (PBPs), which are the principal targets of beta-lactam antibiotics. Over the past years, my research has been funded by the BBSRC, MRC and EC. More recently, I have received a Wellcome Trust Senior Investigator Award (2014-2018) to study peptidoglycan synthesis and degradation in Gram-negative bacteria.

Remarkably, although PBPs are known since the 1960s, we know very little about how they work in the cell to enlarge the peptidoglycan layer when a bacterial cell is growing and dividing. We also do not know key aspects of PBPs catalytic activities, including for example their precise substrate binding sites and modes of processivity. One of our approaches is to reconstitute PBPs in the test tube together with their interaction partners and regulators, which we have identified over the past years, to determine how PBPs are functioning are regulated at the molecular level. For this work we apply various in vitro peptidoglycan synthesis assays using different substrates, including native lipid II of different chemotypes (i.e. the different Gram-positive/-negative versions) and radioactive or fluorescent labelled lipid II. We have established the first useful assay to measure the glycosyltransferase and transpeptidase activities of PBPs with lipid II using an endpoint HPLC-based methodology, and we use a continuous assay for glycosyltransferase activity with fluorescence lipid II substrate. We also use water-soluble precursors such as UDP-MurNAc pentapeptide to assay carboxypeptidase activity.

All this work is only possible due to the availability of the adequate peptidoglycan substrates, the production of which is not trivial for several reasons. First, there is need for a range of substrates for the different enzymes, and the chemistry of the substrates must fit to bacterial species the enzyme was isolated from. For example, PBPs from the Gram-positive pathogen *Streptococcus pneumoniae* require lipid II with an amidated glutamate residue at position 2 and a lysine at position 3 of the stem peptide, and they would not be active on Gram-negative-type, unamidated lipid II. Second, many assays require modified substrates with radioactive or fluorescent labels, and other modifications are extremely useful to gain insights into the catalytic mechanisms of PBPs. Third, although a small amount of a particular particular peptidoglycan substrate may be produced in research laboratories for a pilot experiment, it is a challenge to scale up the production to obtain sufficient amounts for more extensive studies and screens for inhibitors. Thus, the synthesis of the various peptidoglycan substrates in sufficient amounts for basic research and screening purposes can be only a task for a dedicated facility such as BACWAN.

BACWAN is a world-wide unique facility that has been crucial for providing precursors to the bacterial cell wall community and for the recent progress in the biochemistry of cell wall enzymes, which has been published in numerous high-profile papers. In my own work I have used precursors from the BACWAN facility for projects funded by the BBSRC, MRC and EC, and I need these precursors for my ongoing Wellcome Trust project. I therefore strongly support your application to the Wellcome Trust to continue the BACWAN facility and expand its synthesis capacity to the benefit of the bacterial cell wall research community.

With best regards,

Waldemar Vollmer

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12 February 2015

Dear David

My laboratory has been engaged in research on Bacterial Secretion Systems for many years. We have made important contributions in two particular secretion systems: i- the chaperone-usher pathway, based in the outer membrane of Gram-negative bacteria and involved in making and secreting bacterial pili and ii- the type IV secretion system (T4SS), which span both the inner and outer membranes of Gram-negative bacteria and are involved in secreting both DNAs and proteins. Both systems play important roles in bacterial pathogenesis, the first being crucial in adhesion of bacterial cells to the host, and the second being involved not only in secreting lethal toxins and transforming proteins but also in the spread of plasmid-encoded antibiotics resistance genes. We have started to exploit our structural work to design novel inhibitory compounds for these systems, which, we hope, might be developed as antibacterials in the future. Both the systems we study being embedded in membranes, we have a keen interest in understanding how they cross the peptidoglycan layer.

It is therefore with great enthusiasm that I write in support of your application to the Welcome Trust for support for the BACWAN peptidoglycan reagent facility. Whilst I have/have not used the facility in the past I understand that this facility can support global research in the area of bacterial cell wall biosynthesis. Development of the synthesis facility at Warwick has enabled widespread access to these key reagents that are not available commercially and will open up new areas of research. Such a

facility is extremely important and timely in a period where global efforts to tackle antimicrobial resistance is of paramount importance and the cell wall is a proven target for antimicrobial therapy.

Yours sincerely,

Gabriel Waksman

Université de Montréal

Faculté de médecine
Département de microbiologie, infectiologie et immunologie

Montreal, February 12th, 2015

To whom it may concerns,

I am writing this letter to strongly support the BACWAN facility at the University of Warwick. **The continuation of the BACWAN facility at the University of Warwick is essential to foster both fundamental research in the area of bacterial peptidoglycan biosynthesis and to allow the use of the enzymes involved in peptidoglycan biosynthesis for the development of new antibacterial agents.**

As a PhD student in the laboratory of Roger C. Levesque at Université Laval, Quebec, Canada, the collaboration with the University of Warwick for the acquisition of the nucleotidic substrates of the Mur ligases required for the assembly of the peptidic moiety of the peptidoglycan was crucial for my work. The availability of these substrates allowed me to characterize the first peptide inhibitors of these essential enzymes in the pathogenic bacterium *Pseudomonas aeruginosa*; one of the main causes of nosocomial infections and the leading cause of death in cystic fibrosis patients. This work was published in the journals Peptides, BMC Biochemistry and the Biochemical Journal. **Now as an assistant professor at the Université de Montreal, two main projects of my research program depend on the availability of peptidoglycan intermediates at the BACWAN facility at the University of Warwick**. I am aiming at identifying multi-target inhibitors of the Mur ligases to develop a new class of antibacterial agents with a decreased probability of resistance development. My lab recently published a review in Molecular Microbiology on the advantages of this strategy. I also plan on using a similar strategy to find multi-target inhibitors of the penicillin-binding proteins. For this purpose, I will require Lipid II from the BACWAN facility. These projects are supported by the Université de Montréal and the FRQS governmental funding agency**. I thus have graduate students that depend on the availability of the substrates from the BACWAN facility for their respective projects.**

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The biochemical pathway for peptidoglycan assembly is one of the bests and more validated sources of targets for antibacterial development. They are essential for bacterial survival, growth and pathogenesis. They are conserved in a key spectrum of pathogenic bacteria but are absent in eukaryotic cells. Indeed, many of our most successful antibiotics target peptidoglycan assembly, such as penicillin and vancomycin. However, bacterial resistance to antibiotics is one of the most critical public health concerns and the scientific community must develop new antibacterial agents. In fact, this problematic involves all bacterial pathogens and all clinically useful antibiotics, complicating the treatment of infectious diseases and increasing human morbidity, mortality and health care costs. **To allow the development of new antibacterial agents targeting the peptidoglycan biosynthesis pathway and protect human health against the problematic of bacterial resistance to antibiotics, the BACWAN facility at the University of Warwick that provides peptidoglycan intermediates required for the study and inhibitor development against peptidoglycan biosynthesis enzymes must stay open.**

Sincerely,

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