ANEXO

1.1. Justification and expected contribution of the project to solving specific problems linked to the selected thematic priority

The consequences of antimicrobial resistance (AMR) are already severe, and without intervention, bacterial infections are poised to resurface as a predominant global health concern. Compounding this issue is the near absence of a viable pipeline for the development of new antibiotics in clinical stages. There is a pressing demand for a molecular-level understanding of essential bacterial mechanisms in multidrug-resistant (MDR) pathogens. This knowledge can serve as a foundation for identifying novel targets, enabling the development of a new class of antibacterials with distinct mechanisms of action that reduce the likelihood of resistance emergence. **The pertinence and innovative aspects of the BW2CARE research program**, hence, rely on the following elements: 1) The use of **highly relevant model organisms** such as *S. pneumoniae*, *P. aeruginosa,* MRSA and *C. difficile;* 2) The **innovative findings** made in the BW2CARE (summarized in **Table 1**) that lay the fundament for the proposed research and 3) **Multidisciplinary approaches** obtained by bringing together the PI with world-leading groups specialized in microbiology, biochemistry, and drug discovery with different and complementary expertise of relevance to drug discovery.

Table 1. Main aspects of BW2CARE proposal that go beyond current state-of-the-art

Beyond state-of-the-art	Applications and Impact	RO
Delineating mechanisms by which essential FtsEX system controls bacterial cell division	Fundamental knowledge about a new unexplored target to kill bacteria and information on target interaction networks.	1
Molecular basis of regulation of dormancy en C. difficile	Development of new molecules disrupting the germination of spores in recalcitrant C.d pathogen.	2
Detailed structural information on the signaling events (Mec/Bla operons) involved in intrinsic β-lactam resistance in MRSA and C. difficile	Hits that alone or in combination with β-lactams have activity against drug-resistant MRSA and C. difficile.	3
Structural and functional characterization of the initial steps triggering competence and virulence in S. pneumoniae.	Identification of a novel target to combat virulence in pneumococcal infections.	3
Tackling stability of OM barrier in G(-) MDR pathogens.	Our work will allow the designing of new inhibitors destabilizing OM acting in synergy with actual β-lactam antibiotics.	4
Detailed molecular description of the PG recycling process and the link with AMR	Optimization of naturally synthetized inhibitors (i.e. Bulgecin A) applicable in combination with conventional antibiotics therapy with potential high efficacy.	4

1.2. *General and Specific objectives.*

The **BW2CARE** project aims to offer a deeper understanding of the dynamics and regulatory mechanisms governing bacterial cell wall integrity in multidrug-resistant (MDR) pathogens. These mechanisms are of utmost importance in both fundamental research and practical applications, given their direct involvement in antimicrobial resistance. The general Research Objectives (RO) are:

- **RO1.** *Regulation of PG hydrolases in bacterial cell division.*
- **RO2.** *Mechanism of spore germination in Clostridioides difficile.*
- **RO3.** *Signaling mechanisms on the cell wall that trigger virulence and resistance.*
- **RO4.** *PG remodeling and antibiotics resistance mechanisms in MDR pathogens.*

1.3. Previous results of the team in the theme of the proposal.

The group headed by J.A. Hermoso has a solid experience of more than 35 years in the macromolecular crystallography field. This experience is reflected in more than 207 papers in Structural Biology (see the full list of the papers of the PI), more than 247 crystal structures deposited in PDB, and 35 projects (23 of them as PI). This long

trajectory results in a strong background in all the techniques needed for structural determination by crystallography: from production, purification, crystallization, phasing, X-ray sources, to structural analysis. Our group has a unique expertise in the field of structural and functional characterization of bacterial proteins involved in virulence and AMR. Our projects intend a profound characterization of each system in a multidimensional approach (biochemical, molecular and biomedical) and therefore a large network of collaborations with many group leaders worldwide has been established.

International Competitive Funding Relevant to the Project. The PI has been granted with different international grants related to some topics connected with the project:

- *EU Grants*:
  2005-2008: *Innovative Tools for Membrane Structural Proteomics.* STREP Project, Sixth Framework Programme- EU (513770); **1.9 M€.**
  2009-2011: *Combating Antibiotics Resistant Pneumococci by Novel Strategies Based on in vivo and in vitro Host-Pathogen Interactions.* Collaborative Project, Seven Framework Program- (EU-CP223111); **3 M€.**
  2024-2027: *Cell Envelope Antibacterials "CLEAR".* HORIZON-MSCA-2022-DN-01 (Grant 101119534). **2.7 M€**
- USA *NIH Grants*:
  2010-2015: *Novel Oxadiazols for the Treatment of Drug-Resistant Gram-Positive Bacteria.* (1R01AI090818-01); **5.4 M$**
  2015-2020: *The Quinazolinone Class of Antibacterial Agents*. (1R01AI116548); **0.6 M$**
- *Swiss National Science Foundation:*
  2021-2024: *Origins of Broad-Spectrum Beta-Lactam Resistance: Multidimensional Dissection of Chromosomally Encoded Metallo-Beta-Lactamases*. SINERGIA Grant (CRSII5_198737/1). **2.2 M CHF**. IPs: P. Viollier (Univ. Geneva), J.A. Hermoso (CSIC) and M. Dal Peraro (EPF Lausanne)

The topics of the SINERGIA grant (evolution of β-lactam resistance conferred by chromosomally encoded broad-spectrum metallo-β-lactamases) and the CLEAR grant (training the next generation of researchers to develop novel solutions to fight drug-resistant Gram-positive bacteria) do not overlap with objectives of BW2CARE.

1.4. ***Training program planned in the context of the requested Project*.** We intend to provide a wide range of skills development targeted to prepare students for a successful research career and generate a community for the exchange of ideas and experiences within our research associates. Our program is based on:

(i) Advanced courses to deepen scientific knowledge. The student will follow different courses and workshops in the structural biology field. One of them is the international Macromolecular Crystallography School (http://www.xtal.iqfr.csic.es/MCS223/) we organize. The school program covers aspects such as sample preparation, crystallization, phasing, modeling, crystallographic refinement, and analysis of the structural results.

(ii) Experimental experience in different laboratories. We are keen that students gain early experience in the laboratory and in varied laboratory environments. We also intend stages at the laboratories from our collaborators worldwide (Mobashery USA, Hammerschmidt Germany, De Bolle Belgium).

(iii) Scientific communication. We have very frequently meetings with our international collaborators in which students need to present and to discuss her/his work. We also promote participation in national and international meetings in the field.

(iv) Doctorate Program. Student will follow her/his doctorate in “Programa de doctorado en Biociencias moleculares” at Univ. Autónoma de Madrid or in “Programa de doctorado en bioquímica, biología molecular y biomedicina” at Univ. Complutense de Madrid.

(v) Marie Sklodowska-Curie Action. The overarching aims of the CLEAR program are to train a future generation of outstanding researchers in reacting to the antimicrobial resistance (AMR) challenges with innovative approaches benefitting society, academia, and industry. All the activities and meetings inside the consortium will be available for the group members who will benefit from this outstanding environment centred on AMR.