BCL-2/IP3R CHANNEL STRUCTURAL DYNAMICS UNDERLYING COMPLEX FORMATION, FUNCTION AND DISEASE (BINAMICS)

DMP TITLE

ADMIN DETAILS

Project Name: Bcl-2/IP3R channel structural dynamics underlying complex formation, function and disease (BInamics) - DMP title
Project Identifier: G094522N
Grant Title: G094522N
Principal Investigator / Researcher: Geert Bultynck
Description: Anti-apoptotic Bcl-2 proteins not only act the mitochondria where they prevent the release of pro-cell death factors but also function at the endoplasmic reticulum (ER), the organelle that serves as the main calcium-storage site in mammalian cells. At the ER, Bcl-2, via its "BH4 domain", directly inhibits the IP3 receptor, a large calcium release channel, preventing mitochondrial Ca2+ overload and thus cell death to occur. Previous studies indicated that Bcl-2's "BH4 domain" targets two regions in the IP3R channel. Recent cryoEM studies revealed that these Bcl-2-binding sites are located on the top of the channel structure, in principle out of reach for Bcl-2's BH4 domain. Thus, it is not clear how Bcl-2, being a "dwarf protein", through its BH4 domain can inhibit IP3R channels, giant channels. Using an interdisciplinary approach combining biophysics, biochemistry & cell biology with structural dynamics of proteins (BInamics), we aim to unravel the mechanisms by which Bcl-2 controls IP3Rs activity. Moreover, phosphorylation and disease associated mutations, including Bcl-2 mutations that recently emerged in leukemia patients who were under prolonged venetoclax treatment, are present in Bcl-2, but their impact on Bcl-2's properties to modulate IP3R function remain unknown. Through BInamics, we aim to elucidate their impact on IP3R/Bcl-2 complexes.
Institution: KU Leuven

1. GENERAL INFORMATION

Name applicant
Supervisor Spokesman: Bultynck Geert
Co-Supervisor: Karamanou Spyridoula (Lily)
Co-Supervisor Yule David I

FWO Project Number & Title
G094522N

Bcl-2/IP3R channel structural dynamics underlying complex formation, function and disease (BInamics)

Affiliation
  ● KU Leuven
  ● Other

KU Leuven:
  ● Bultynck Geert (Laboratory of Molecular & Cellular Signaling (LMCS))
  ● Karamanou Spyridoula (Lily) (Research Manager (BAP)) (Laboratory of Molecular Bacteriology (LMB) - Rega Instituut)

Rochester University (NY):
  ● Yule David I (Professor) (School of Medicine and Dentistry)

2. DATA DESCRIPTION

Will you generate/collect new data and/or make use of existing data?
  ● Generate new data

Describe in detail the origin, type and format of the data (per dataset) and its (estimated) volume. This may be easiest in a table (see example) or as a data flow and per WP or objective of the project. If you reuse existing data, specify the source of these data. Distinguish data types (the kind of content) from data formats (the technical format).
For LMCS:
Biological material: cell lines, plasmids, primers, peptides,antibodies, purified proteins,...
Experimental data:
  ● Western blots: .scn (imagelab files), TIFF
  ● Fluorescent microscopy data: ziar, zistream, zvi (Zeiss) and ....(Nikon), txt files
  ● Flexstation analysis: .pda (softmax pro) and .txt
  ● FDSS µcell screening: excel files, .txt
  ● OpenSPR, BLI MST: .txt
  ● 45Ca2+ unidirectional flux experiments: .rtf and .xlsx
  ● Cell death measurements via FACS analysis as .fcs (flow jo files).
  ● Cell death/cell proliferation experiments via Incucyte analysis are obtained as incucytezoom files and saved as .xls, .tif and .mp4
  ● DNA Sequences and all relevant information is stored in a database
  ● Cell line datafiles will be preserved in the inventory management section of an electronic lab notebook and linked to the experiments.
  ● The data in the ELN is stored in a database.

Both the raw data and the processed data of experiments including graphs will be preserved, as well as the resulting manuscripts. Analysis of the various techniques and storage of overall lab data will be in electronic notebooks or on the network drives of the university. Please note that the volume of most data is not going to be large enough to cause problems with our storage capacity. For the microscopy data we

only will keep the original .ziar or ..... and .txt files for long term storage as these are sufficient to generate the other files.

3. LEGAL AND ETHICAL ISSUES

Will you use personal data? If so, shortly describe the kind of personal data you will use. Add the reference to your file in KU Leuven's Register of Data Processing for Research and Public Service Purposes (PRET application).   Be aware that registering the fact that you process personal data is a legal obligation.
- No

Privacy Registry Reference:

Short description of the kind of personal data that will be used:

Are there any ethical issues concerning the creation and/or use of the data (e.g. experiments on humans or animals, dual use)? If so, add the reference to the formal approval by the relevant ethical review committee(s)
- No

Does your work possibly result in research data with potential for tech transfer and valorisation? Will IP restrictions be claimed for the data you created? If so, for what data and which restrictions will be asserted?
- Yes

This research project is more fundamental in nature. If this would be the case we will evaluate this on a case by case basis in collaboration with LRD.

Do existing 3rd party agreements restrict dissemination or exploitation of the data you (re)use? If so, to what data do they relate and what restrictions are in place?
- Yes

Some cell lines or other biological materials (e.g. probes) that we may want to use in the progress of this project can be covered by an MTA.

4. DOCUMENTATION AND METADATA

What documentation will be provided to enable reuse of the data collected/generated in this project?
For LMCS:
According to good laboratory practices, each researcher involved in the project, provides detailed descriptions of his/her experimental data acquisition and/or on the generation of new biological materials in his/her electronic laboratory notebooks as appropriate, thereby cross-referring any paper notes that might be used to the electronic files containing the data, and to the biological samples used.

Protocols and products used are indicated and cross-referenced in the laboratory notebooks. Each experiment in the ELN will contain all data files with the exclusion of the microscopy data file (this will be stored on an external drive and cross-referenced) a metadata file with information about the specific dataset or links to other datasets including unique identifier numbers is included in the ELN. Revision history is maintained. JSON formatted files with metadata are included in the ELN.

The researchers involved will store work files on the J-drive or the KU Leuven Enterprise onedrive instead of his/her own laptop hard drive to prevent loss of data.

Cell lines will be documented in a standardized way inside the LMCS cell line database (format: .xlsx; location: J-drive) Write access only by the personnel affiliated to our cell culture facility.

Plasmids will be documented in the ELN database. Information including DNA sequences of primers or other constructs or amino acid sequences of peptides generated or obtained during the project: .txt and .docx files and in ELN.

(LMB) - Rega Instituut):

Each researcher records in detail experimental conditions (with references to plasmids and bacterial strains used) and pairs them with raw data files. Experimental condition files (xls, word), raw data (DAT) and analyzed data (xls, csv) files of one experiment are linked and copied in OneDrive-KULeuven, Dropbox and 2 external hard disks (6TB each) provided by the lab; plus, extra copies on personal laptops. The raw data files are independently backed-up by the lab manager in external hard drives, once per month.

Bacterial strains, plasmids, antibodies and chemicals are documented in lab databases. Access to data files and databases is only permitted to lab members. A copy of data can be provided to coworkers upon request.

For Rochester:

All laboratory members involved in the project document detailed descriptions of their experimental methods in an electronic notebook daily. In the notebook detailed experimental protocols are described, specific references to plasmids, cell lines and chemicals are included together with a description of the data obtained and cross references with Yule laboratory central database records of plasmids cell lines and chemicals stored electronically.

Data files in the form of excel spreadsheets (for patch clamp, optical patch clamp and Calcium imaging) or images (immunoblots, gels, Calcium imaging) are stored on external hard drives and backed up to the University of Rochester "BOX" cloud based storage system. Coworkers from LMCS will be given access privileges. Metadata files will similarly be stored on BOX together will information related to peer reviewed publications.

Will a metadata standard be used? If so, describe in detail which standard will be used. If no, state in detail which metadata will be created to make the data easy/easier to find and reuse.

- Yes

For LMCS:

Metadata will be used. During and after the project all data is available on the LMCS-shared network J-drive and/or via the laboratory notebooks of the researchers involved. This will make the data available to

researcher within the research group. Data and all other information related to peer-reviewed publications will, at present, be archived on the LMCS Archive K-drive (1 TB presently available, can be expanded whenever necessary) as soon as possible after publication.

We will in future also make use of the KULeuven research data repository, launching in 2022. Data will be prepared according to the guidelines set by the university.

(LMB) - Rega Instituut):

Metadata will be used. All data can be readily available during and after the project to people within the lab or to coworkers upon request. The general audience can access the data after publication upon request. We will use the KULeuven data repository once available, following KULeuven guidelines.

5. DATA STORAGE AND BACKUP DURING THE FWO PROJECT

Where will the data be stored?

How is backup of the data provided?
For LMCS:

All data is stored on network drives of the KU Leuven (J-drive, K-drive) with automatic backup at least once per day for these drives by ICT. The electronic lab notebooks are backed up once a day. The individual researcher will also back-up experiments of the ELN to their respective onedrive of the KULeuven as a secondary back-up option.

(LMB) - Rega Instituut):

All data are backed up by individuals on a daily basis (personal and lab copies) on laptops, Dropbox, OneDrive-KULeuven and external hard disks/passports. Additionally, raw data files (from all instruments, common computers) are backed up independently once a month by the lab manager.

For Rochester:

Electronic notebooks on personal laptops are backed up to BOX daily.

Is there currently sufficient storage & backup capacity during the project? If yes, specify concisely. If no or insufficient storage or backup capacities are available then explain how this will be taken care of.
   ● Yes
For LMCS:
Available (drives can be expanded whenever needed):
   ● ELN: 800GB space, currently 5GB
   ● used research group networked-drive: 1TB space, currently 700GB used
   ● K-drive: 1TB space (for archiving purposes)
   ● Each individual researcher: Professional Onedrive (2TB)

For REGA:

Each Researcher gets from the lab a 1-6TB external hard disk, a Dropbox account (2TB), a OneDrive-KUL account (95GB), plus up to 1TB space in every common LAB computer. Additional space is provided upon request. The Lab maintains 4 x 10 TB disks independently of the ones that are the users' responsibility to back up the current raw data.

For Rochester:

We currently have 5 TB space for the laboratory on BOX.

What are the expected costs for data storage and back up during the project? How will these costs be covered?

For LMCS:

ELN: €121 / 1TB J-drive: €51.9 / year/ 100GB K-drive: €156 / year / 1TB MySQL: €41.41 / year
Reparation or replacement costs for cell containers, freezers or other hardware will be covered by the allocated 'consumables' budget of the projects ongoing in LMCS

(LMB) - Rega Instituut):

Each external hard disk costs 120-200 euros, OneDrive-KUL is provided by KUL, Dropbox accounts cost 110-180 euros /year. Such costs are covered by ''consumables'' budgets of ongoing lab projects/grants. The cost of one external hard disk (4TB) may be charged on the ''consumable'' budget of the current FWO project, if the need arises.

For Rochester:

This cost is covered by the University of Rochester since this is defined as financed by "research overhead".

Data security: how will you ensure that the data are securely stored and not accessed or modified by unauthorized persons?

For LMCS:

Every researcher has his/her own login on the ELN, password, and 2FA protected. Access to the J-drive and professional one-drive is via password and 2FA.
Archived material on the K-drive will only be accessible to the lab coordinator and the PI's via u-number and password. Modifications of the archived data are not possible, write privileges are only for the lab coordinator, a read-only privilege only for the PI's. Removal of data is only possible by the ICT department upon request by the lab coordinator and agreement of the PI's.

(LMB) - Rega Instituut):

Direct access to the data is allowed only to lab members. A copy of data can be provided to external coworkers upon request. Each researcher carries his/her own copy of data independent of the copies

made for the lab, plus one copy of raw data remains at all times secured in the PIs office; access to this copy is not allowed even to lab members and is being updated by one dedicated person only.

6. DATA PRESERVATION AFTER THE FWO PROJECT

Which data will be retained for the expected 5 year period after the end of the project? In case only a selection of the data can/will be preserved, clearly state the reasons for this (legal or contractual restrictions, physical preservation issues, ...).
All obtained/generated data will be preserved for at least 5 years (as well the original data as the processed data), as well in Leuven as at our external partner.

Where will the data be archived (= stored for the longer term)?
All data underpinning publications (original and processed data), all accompanying information and the files submitted for publication will be archived in a systematic way on the KU Leuven network K-drive or the KU Leuven research data repository.
The data will be stored on the university's central servers (with automatic back-up procedures) for at least 10 years, conform the KU Leuven RDM policy.

What are the expected costs for data preservation during the retention period of 5 years? How will the costs be covered?
All obtained/generated data will be preserved for at least 5 years (as well the original data as the processed data), as well in Leuven as at our external partner. See questions above about costs involved.

7. DATA SHARING AND REUSE

Are there any factors restricting or preventing the sharing of (some of) the data (e.g. as defined in an agreement with a 3rd party, legal restrictions)?
  ● Yes. Specify:
Data potentially leading to patent application or important for future applications will not be made available or only under restricting conditions.

Which data will be made available after the end of the project?
Preliminary data will be presented in seminars and at national and international meetings as poster/oral communications/invited lectures.
Definitive data will be published in peer-reviewed, international journals (Open Access as per KU Leuven policy). Restrictions as mentioned in previous point.

Where/how will the data be made available for reuse?

All data will be published in academic-peer reviewed journals as soon as possible (for restrictions see above). We aim to publish open access according to KU Leuven policy and publications will be available via Lirias 2.0. Data from published papers will in future be deposited in the KU Leuven research data repository.

When will the data be made available?
- Upon publication of the research results

Datasets will be uploaded to the university research data repository upon publication.

Who will be able to access the data and under what conditions?
- Publications (open access).
- For published data: Via the KULeuven research data repository, conditions to be determined depending on data gathered during the project. Guidelines of the university will be applied.
- For unpublished data: only the PIs and researchers involved (or their scientific collaborators who will continue and follow up on the research after the completion of present project).

What are the expected costs for data sharing? How will the costs be covered?
Publication costs (Open Access) will be covered by the consumables budget. There is no cost involved at the moment for using the KULeuven data repository. 50GB available per researcher per year for free.

8. RESPONSIBILITIES

Who will be responsible for data documentation & metadata?
All researchers involved in the project are responsible for their own part. The Lab managers in the research groups involved will supervise this process.

Who will be responsible for data storage & back up during the project?
All researchers involved in the project are responsible for their own part. The PI's will supervise this process and deal with the long-term storage of data sets.

Who will be responsible for ensuring data preservation and reuse ?
The PI' mentioned in this grant

Who bears the end responsibility for updating & implementing this DMP?
Geert Bultynck