# DMP du projet "MAgnetic Vesicle Rotation Induced Cell Killing"

*Plan de gestion de données créé à l'aide de DMP OPIDoR, basé sur le modèle "ANR - DMP template (english)" fourni par Agence nationale de la recherche (ANR).*

## Plan Details

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| **Plan title** | DMP du projet "MAgnetic Vesicle Rotation Induced Cell Killing" |
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| **Identifier type** | Local identifier |
| **License** | **Name** Creative Commons Attribution Non Commercial Share Alike 4.0 International<br>**URL** http://spdx.org/licenses/CC-BY-NC-SA-4.0.json |
| **Associated documents (publications,reports, patents, experimental plan...), website** | • ANR MAVERICK website : https://www.lcpo.fr/anr-maverick |

## Project Details

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| **Project title** | MAgnetic Vesicle Rotation Induced Cell Killing |
| **Acronym** | MAVERICK |
| **Abstract** | **MAVERICK or the "Nanoblender" project to destroy the tumoral cells**<br>The project investigated the embedding of iron oxide magnetic nanoparticles (MNPs) within the membrane of nanoscale vesicles called “magnetic polymersomes”. Then a magnetic field (at first static) was applied to deform those objects, then their membrane was “cross-linked” so as these vesicles stay elongated. Finally, they were incubated with cancer cells, and then a rotating |

magnetic field was applied to disrupt the cellular membranes.

***The elementary “bricks”: amphiphilic block copolymers, which are macromolecules made of different chemical sequences, with varying chemical natures and affinities towards water.***

These magnetic polymersomes were designed to act as nanometric mechanical devices inside cancer cells that could fight tumoral growth. While they have been mainly studied for their ability to generate heat and treat cancer by so-called magnetic hyperthermia, this research focuses on their shape change under an applied magnetic field, and ability to retain such elongated shape. The project goal was first to create such elongated magnetic polymersomes, then their rotational motion by a rotating magnetic field to kill cancer cells by mechanical disruption. To achieve this, we introduced new types of amphiphilic copolymers able to form vesicles, and we added magnetic nanoparticles within their membranes. More precisely, we studied two copolymer architectures: the first one is a linear ABC triblock structure where A is poly(ethylene glycol) or PEG, a common biocompatible hydrophilic polymer), B is poly(trimethylene carbonate) or PTMC and C is polyisoprene or PI, B and C being both rubbery (*i.e.* soft and fluid) hydrophobic polymers. The second one is a graft copolymer AB-*graft*-C, where the polyisoprene chains were introduced as pendant groups instead, like the teeth of a comb, to increase their degree of entanglement in the membranes and thus of mechanical rigidity after photo-crosslinking.

***Forming the polymersomes, small soft shells encapsulating an aqueous core and magnetic nanoparticles in their membranes, and study of static magnetic field induced shape change.***

The polymersomes were formed by the process called “self-assembly”, based on relative affinities of hydrophilic blocks for water and of hydrophobic blocks for the MNPs, themselves grafter with hydrophobic molecules. Then applying a magnetic field induces alignment of the MNPs, which causes the polymersomes to change their shape, becoming elongated ellipsoids. The final shape was made stable by cross-linking (*i.e.* chemical bond formation) under UV light of the C=C double bonds of polyisoprene previously made more reactive by changing a small fraction of them as epoxide rings (like for epoxy adhesive glue).

After the creation of these magnetic polymersomes and study of their deformation, the project aimed to demonstrate their potential as mechanical devices for causing cell death. To do so, pancreatic cancer cells were incubated with field-induced elongated and cross-linked polymersomes, then a rotating magnetic field was applied with frequency in the 0.5-10 Hz range. Optimal efficacy in terms of cell proliferation decrease was found for a frequency of 1 Hz and an intensity B=40 mT. This research provides valuable insights into designing elongated nanoobjects based on biocompatible polymers, and their response to magnetic fields. The study will be followed on, notably by a proof of concept not only through *in vitro* but also *in vivo* assays, before possible introducing a new cancer therapy.

**Funding**

- Agence nationale de la recherche (ANR) : ANR-19-CE09-0024

**Start date** 2019-11-01

**End date** 2023-12-31

**Partners**

- LABORATOIRE DE PHYSIQUE ET CHIMIE DES NANO-OBJETS https://ror.org/042xmz674
- Bordeaux Imaging Center
- Laboratoire de chimie des polymères organiques https://ror.org/056n05x05

**Research outputs :**

1. NMR spectra (Jeu de données)
2. TEM microscopy images (Image)
3. Cell toxicity assays (Jeu de données)
4. Magnetization curves (static magnetization curves and dynamic magnetization hysteresis loops) (Jeu de données)
5. Specific loss power (SLP) also called Specific absorption rate (SAR) measurements (Jeu de données)

**Contributors**

| Name | Affiliation | Roles |
|---|---|---|
| Olivier Sandre - https://orcid.org/0000-0002-1815-2702 | | • Coordinateur du projet<br>• Personne contact pour les données (TEM micrographs, Cell toxicity assays, Specific loss powers, NMR spectra, Magnetization curves)<br>• Responsable du plan |

# DMP du projet "MAgnetic Vesicle Rotation Induced Cell Killing"

## 1. Data description and collection or re-use of existing data

### NMR spectra

**1a. How will new data be collected or produced and/or how will existing data be re-used?**

NMR spectra (proton, carbon, phosphate, 2d-NMR, DOSY...) are collected on the Bruker Avance 400MHz spectrometer at the laboratory of partner 1 of the MAVERICK project (LCPO). The objective is to demonstrate the success and assess the yield of all chemical reactions necessary to produce the block copolymers forming polymersomes for the project, and to functionalize them (with fluorescent dyes, with anchoring moieties to embed magnetic iron oxide nanoparticles in their membranes, etc.)

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**1b. What data (for example the kind, formats, and volumes), will be collected or produced?**

The main software used is Bruker Topspin 4, which license is free for academic use: https://www.bruker.com/service/support-upgrades/software-downloads/nmr/free-topspin-processing/nmr-topspin-license-for-academia.html. Bruker files are propriatory (600 ko/spectrum, 1/day on average), yet this format is used by the community of chemists all over the world.

### TEM microscopy images

**1a. How will new data be collected or produced and/or how will existing data be re-used?**

Transmission electron microscopy (TEM) images and high resolution images (HR-TEM) are produced at the Bordeaux Imaging Center (BIC), partner 3 of the MAVERICK project. The objective is to check the vesicular morphology of samples prepared by a nanoprecipitation route from the copolymers synthesized for the project. Later when the polymersomes will be elongated and chemically crosslinked under a magnetic field, TEM images will be used to asses that their shape does not relax when the field is switched off.

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**1b. What data (for example the kind, formats, and volumes), will be collected or produced?**

The TEM images are acquired with the Digital Micrograph software (GATAN – AMETEK) with their own format .dm3 or dm4. This proprietary format contains the metadata. This is the specific software of the camera and therefore is exclusive. This software used at the BIC for over 15 years is known and widely used by the community. The users can also open the .dm3 and .dm4 files with the open source software FIJI (i.e. ImageJ with plugins to use various microscopy formats https://imagej.net/Fiji) and convert them in other formats (TIFF, JPEG...). Only the scale can be included (in TIFFs).

The .dm3 images are acquired at high resolution (8 MPixels) with a size of 30Mbytes, which can be reduced to 10Mbytes (TIFF files with LZW compression), while keeping the scale. Approximately 30 images are produced per TEM session (1 every 2 weeks).

## Cell toxicity assays

**1a. How will new data be collected or produced and/or how will existing data be re-used?**

Cell toxicity assays (TOX) are performed in the RCTC team of LPCNO (partner 2).

The 1st objective is to assess that the polymersomes alone are not toxic to cells (in absence of applied oscillating or rotating magnetic field) and determine their using conditions (concentrations, incubation times…) to get free of any cell cytotoxicity for further on-demand drug delivery experiments using magnetic field. Different cell lines will be used: fibroblasts, epithelial cells, monocytes, and macrophages (these later ones being the first cells in contact with nanoparticles in *in vivo* experiments). Cytotoxicity will be at first evaluated as a function of dose and time of treatment by MTT assay measuring the cell viability. Preliminary results showed that polymersomes do not present cytotoxicity on HEK293 (human embryonic kidney cell) and Raw 264.7 (murine macrophages) cells up to 50 µg/mL, after 24h incubation.

After the determination of safe conditions, the optimal polymersomes uptake will be evaluated. Then, the aim will be to demonstrate that the application of an alternating or a rotating magnetic field induce the drug release from polymersomes in cells that have internalized anisotropic magnetic polymersomes. Finally, the effects on cell viability, cell mortality and the nature of cell damage, following magnetic field exposure and/or drug release, will be studied.

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**1b. What data (for example the kind, formats, and volumes), will be collected or produced?**

Cell viability will be expressed as percentage of viability of control cells in absence of polymersomes.

Cell mortality will be expressed as percentage of dead cells compared to negative control, as assessed by different assays: Annexin V and/or propidium iodide labeling.

Statistical analysis will be performed using GraphPad software.

## Magnetization curves (static magnetization curves and dynamic magnetization hysteresis loops)

**1a. How will new data be collected or produced and/or how will existing data be re-used?**

Static magnetization curves of suspensions of magnetic iron oxide nanoparticles in buffers and in cell extracts are acquired at the LPCNO (Partner 2) using a superconducting quantum interference device (SQUID) magnetometer. Dynamic magnetization curves under alternating magnetic fields are gathered both at LPCNO on a lab-made instrument (up to 100 kHz) and up to 350 kHz at LCPO on a commercial setup (Advanced AC Hyster from Nanotech Instruments, Madrid, Spain, https://www.ntsol.es).

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**1b. What data (for example the kind, formats, and volumes), will be collected or produced?**

Data will consist in magnetic hysteresis loops (i.e. plots of the magnetization as a function of the magnetic field for increasing and decreasing values), which are analyzed for determining the average and standard deviation values of several magnetic parameters (coercive field, remnant magnetization, maximal magnetization, cycle surface area and SAR) from each measurement, including determination of odd harmonics (amplitude and phase) of M(H) which can be correlated with temperature.

## Specific loss power (SLP) also called Specific absorption rate (SAR) measurements

**1a. How will new data be collected or produced and/or how will existing data be re-used?**

The heating efficiencies of magnetic NPs suspended in buffers or in cell extracts (SAR or SLP) under applied alternating magnetic fields are acquired up to 755 kHz at LCPO (Partner 1) and 350 kHz at LPCNO (Partner 2) by calorimetric measurements following recommendations of the "white paper" on SAR determinations entitled “Challenges and Recommendations for Magnetic Hyperthermia Characterization Measurements” to appear in International Journal of Hyperthermia (2021) written by a large consortium of COST TD1402 action RadioMag members http://www.cost-radiomag.eu).

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**1b. What data (for example the kind, formats, and volumes), will be collected or produced?**

Raw data will be supplied as numerical tables (i.e. profiles of temperature vs time for calorimetric measurements or magnetization vs applied magnetic field for AC magnetometry). Examples of temperature profiles are given on: https://doi.org/10.5281/zenodo.4281153.

## 2. Documentation and data quality

**2a. What metadata and documentation (for example the methodology of data collection and way of organising data) will accompany the data?**

The data will be accompanied by a “Readme.txt” file with the information on samples and experimental methodology. We have been using specification datasheets of synthesized samples for 10 years, since our participation in FP7 European project NanoTher (www.nanother.eu). We will adapt them according to templates like those developed by the NIH institute on cancer for nanomaterials: https://wiki.nci.nih.gov/display/ICR/ISA-TAB-Nano#ISA-TAB-Nano-title

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**2b. What data quality control measures will be used?**

The experimental data are discussed between the PhD student and her advisors, and confronted to those obtained by the partners. Any source of data inconstancy or experiment error will be analyzed with the engineers in charge of the experimental setups (NMR, TEM, TOX, MAG, SAR) and double-checked by comparison with precision and reproducibility of analogous data reported in literature.

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## 3. Storage and backup during the research process

### NMR spectra

**3a. How will data and metadata be stored and backed up during the research?**

The NMR spectra are saved on an internal server at LCPO. Backups are made on a daily basis. The data are also shared with users using the institutional cloud service: https://mycore.core-cloud.net

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**3b. How will data security and protection of sensitive data be taken care during the research**

The institutional cloud https://mycore.core-cloud.net meets requirements of data protection and safety defined by the organism (CNRS).

## TEM microscopy images

**3a. How will data and metadata be stored and backed up during the research?**

The TEM images are saved on an internal server at BIC during session. After the session, the image are saved and transferred to the users by https://filesender.renater.fr/ so they can copy them on their hard disks and on institutional cloud service https://mycore.core-cloud.net (after compression into LZF TIFF files to minimized disk space).

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**3b. How will data security and protection of sensitive data be taken care during the research**

The institutional cloud https://mycore.core-cloud.net meets requirements of data protection and safety defined by the organism (CNRS).

## Cell toxicity assays

**3a. How will data and metadata be stored and backed up during the research?**

The institutional intranet server "i2mc-share.inserm.lan" of the RCTC team (Partner 2) meets requirements of data protection and safety defined by the organism (CNRS).

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**3b. How will data security and protection of sensitive data be taken care during the research**

Data security is handled by the institutions of the partner laboratories (Bordeaux INP, CNRS, University of Bordeaux, University of Toulouse Paul Sabatier, INSA Toulouse, and INSERM).

## Magnetization curves (static magnetization curves and dynamic magnetization hysteresis loops)

**3a. How will data and metadata be stored and backed up during the research?**

The MAG curves are saved on an internal server at LCPO and LPCNO. Backups are made on a daily basis. The data are also shared with users using the institutional cloud service: https://mycore.core-cloud.net

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**3b. How will data security and protection of sensitive data be taken care during the research**

Data security is handled by the institutions of the partner laboratories (Bordeaux INP, CNRS, University of Bordeaux, University of Toulouse Paul Sabatier, INSA Toulouse, and INSERM).

### Specific loss power (SLP) also called Specific absorption rate (SAR) measurements

**3a. How will data and metadata be stored and backed up during the research?**

The SAR curves are saved on an internal server at LCPO and LPCNO. Backups are made on a daily basis. The data are also shared with users using the institutional cloud service: https://mycore.core-cloud.net

**3b. How will data security and protection of sensitive data be taken care during the research**

Data security is handled by the institutions of the partner laboratories (Bordeaux INP, CNRS, University of Bordeaux, University of Toulouse Paul Sabatier, INSA Toulouse, and INSERM).

## 4. Legal and ethical requirements, code of conduct

**4a. If personal data are processed, how will compliance with legislation on personal data and on security be ensured?**

No personal data processed.

**4b. How will other legal issues, such as intellectual property rights and ownership, be managed? What legislation is applicable?**

The data belong to the three partners of the projects: LCPO, LPCNO and BIC. They are all research units associated to the same institution (CNRS), which defines its IP politics in agreement with the other institutional partners (University of Bordeaux, Bordeaux INP, INSERM, University of Toulouse Paul Sabatier, INSA Toulouse).
Whenever possible and meaningful, results will be submitted to institutional IP offices (SATT Aquitaine Science Transfert and Toulouse Tech Transfert) for possible patent filing. Otherwise, data will be shared under Creative Commons Attribution Non-Commercial Share Alike license (CC-BY-NC-SA).

**4c. What ethical issues and codes of conduct are there, and how will they be taken into account?**

The project is conducted in accordance with the charter of scientific integrity and research ethics established in partnership between universities, CNRS, INSERM and INRAE: https://college-doctoral.u-bordeaux.fr/en/News/Scientific-integrity-and-research-ethics

# 5. Data sharing and long-term preservation

**5a. How and when will data be shared? Are there possible restrictions to data sharing or embargo reasons?**

All the routine data are not useful to be shared. However, the characterization data of samples selected for scientific publications will be made available on the long-term thanks to a trustworthy repository.

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**5b. How will data for preservation be selected, and where data will be preserved long-term (for example a data repository or archive)?**

The data supporting the results of all published articles will be made available through the journal publisher websites and on a long-term repository such as 4TU.ResearchData https://researchdata.4tu.nl/en/ (using their free 10 Gigabyte space, as no funding was initially planned in the ANR MAVERICK budget).

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**5c. What methods or software tools are needed to access and use data?**

NMR spectra can be read by Bruker Topspin software, which can be freely used by academic researchers https://www.bruker.com/service/support-upgrades/software-downloads/nmr/free-topspin-processing/nmr-topspin-license-for-academia.html

The data points of all graphics (curated data and fitting curves) will be made available as text files (.CSV) for instance using FigShare.com repository.

TEM Images can be read by FIJI and, after conversion into TIFF or JPG format, by most standard imaging softwares (ImageJ, IrfanView, ACDSee...).

GraphPad, is used for statistical analysis of TOX data. KaleidaGraph or Origin plotting software will be used to analyze magnetic properties of polymersomes (Ms values, SAR...) and data fitting with theoretical models.

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**5d. How will the application of a unique and persistent identifier (such as a Digital Object Identifier (DOI)) to each data set be ensured?**

Each data set will be provided a DOI thanks to their proper publication (under FAIR principles of Open Science) using Zenodo.org, FigShare.com and data.4tu.nl/info/en/ or other public repositories.

The list of all available data sets will be provided on the MAVERICK project web page: https://www.lcpo.fr/anr-maverick

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# 6. Data management responsibilities and resources

## NMR spectra

**6a. Who (for example role, position, and institution) will be responsible for data management (i.e. the data steward)?**

Partner 1 (LCPO) is responsible for the data on chemical structures and physicochemical characterizations.

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**6b. What resources (for example financial and time) will be dedicated to data management and ensuring**

**that data will be FAIR (Findable, Accessible, Interoperable, Re-usable)?**

The partners will devote a part of their time on curating the data and publishing them on public repositories, concomitantly with the writing of the publications.

## TEM microscopy images

**6a. Who (for example role, position, and institution) will be responsible for data management (i.e. the data steward)?**

Partner 1 (LCPO) and Partner 3 (BIC) share the responsibility for the curation and sharing of electron microscopy images. The BIC uses specific internal resources dedicated to the implementation, monitoring and security of the TEM images server.

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**6b. What resources (for example financial and time) will be dedicated to data management and ensuring that data will be FAIR (Findable, Accessible, Interoperable, Re-usable)?**

The partners will devote a part of their time on curating the data and publishing them on public repositories, concomitantly with the writing of the publications.
The BIC regularly invests in the development of IT resources in terms of storage and backup.

## Cell toxicity assays

**6a. Who (for example role, position, and institution) will be responsible for data management (i.e. the data steward)?**

The RCTC team of Partner 2 is responsible for the cell toxicity data.

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**6b. What resources (for example financial and time) will be dedicated to data management and ensuring that data will be FAIR (Findable, Accessible, Interoperable, Re-usable)?**

The partners will devote a part of their time on curating the data and publishing them on public repositories, concomitantly with the writing of the publications.

## Magnetization curves (static magnetization curves and dynamic magnetization hysteresis loops)

**6a. Who (for example role, position, and institution) will be responsible for data management (i.e. the data steward)?**

Partner 1 (LCPO) and Partner 2 (LPCNO) share the responsibility for the curation and sharing of electron microscopy

images.

**6b. What resources (for example financial and time) will be dedicated to data management and ensuring that data will be FAIR (Findable, Accessible, Interoperable, Re-usable)?**

The partners will devote a part of their time on curating the data and publishing them on public repositories, concomitantly with the writing of the publications.

## Specific loss power (SLP) also called Specific absorption rate (SAR) measurements

**6a. Who (for example role, position, and institution) will be responsible for data management (i.e. the data steward)?**

Partner 1 (LCPO) and Partner 2 (LPCNO) share the responsibility for the curation and sharing of SAR measurements.

**6b. What resources (for example financial and time) will be dedicated to data management and ensuring that data will be FAIR (Findable, Accessible, Interoperable, Re-usable)?**

The partners will devote a part of their time on curating the data and publishing them on public repositories, concomitantly with the writing of the publications.