université de BORDEAUX

Frontiers of Life
Bordeaux

Frontiers of Life
Post Doc Call 2024

Call for Post-doc positions in Frontiers of Life

Frontiers of life is a Univ. Bordeaux Research Network (RRI) focused on the re-engineering of living systems and artificial cells. It is an interdisciplinary network spanning physics, chemistry, life science and engineering and funded by the Univ. Bordeaux. The current call aims at supporting the activities of our research community and strengthening the international visibility of our research activities.

The present call aims at funding post-doc positions on new research projects aligned with the main objectives of Frontiers of Life.

- The call is open with a deadline on March 15 2024 for the submission of proposals. Applications should be sent to : rri.frontiersoflife.copil@diff.u-bordeaux.fr
- The call is open to the broad community of scientists on the campus of the University of Bordeaux having scientific activities of relevance to the research themes of Frontiers of Life.
- Joint supervision between PIs is preferred but not mandatory.
- Multidisciplinary projects are preferred but not mandatory.
- The duration of the post-doc position can be 12, 18 or 24 months.
- The funding will only cover personnel costs. Details must be given on the plan of the PIs to cover or experimental costs, to demonstrate feasibility.
- **/!** For the accepted projects, the details of the hiring procedures will be explained to the laureates in the decision letter. The position will be centrally funded by the RRI.

All proposals will be evaluated within the steering committee and ranked according to the following criteria:

- Scientific quality of the proposal, its objectives and feasibility of the project: 50%
- Added value for Frontiers of Life and alignment with the objectives of Frontiers of Life, long term perspective of the project: 30%
- Quality of the researchers involved in the supervision and of the organization implemented, capacity to supervise: 20%

1. Overview

PROJECT TITLE	**Shaping lipid membranes via chemical signals**
PROJECT ACRONYM	**ARCHEM**
DURATION OF THE POSITION (IN MONTHS)	**24 MONTHS**
NAME OF THE PRINCIPAL INVESTIGATORS	**Assist. Prof. Laura Alvarez-Frances**
LABORATORY (MAIN HOST)	**Centre de recherche Paul Pascal UMR5031**
LIST OF THE PARTNERS AND LAB OF THE PARTNER	**Prof. Andela Saric (ISTA Austria)**

2. Description (2 pages max)

2.1 Context & state of the art

State of the Art

Living cells exhibit complex interaction pathways to respond and adapt to their environment. In particular, interactions with the environment via membrane protrusions facilitate locomotion[1], sensory[2] and communications processes[3]. The significance of protrusions extends to eukaryogenesis (evolution of eukaryotic cells from prokaryotic ancestors[4]), with recent theories are pointing to the importance of membrane deformations as sources of evolutionary innovation for 'complex' organisms (e.g., eukaryotes)[5]. At an earlier evolutionary stage, protocells - considered precursors to living cells[6], might have transitioned from non-living to living units by benefiting from similar boundary interactions. Membrane-bound protocells - such as lipid vesicles - emerge as spatially confined micro-sized compartments from the self-assembly of phospholipids[7], establishing chemical environments separated from the surrounding aqueous medium. Recent efforts have focused on out-of-equilibrium processes driven by environmental energy and matter intake, as a crucial step in transitioning from protocells to self-sustainable life-like units[8]. ***Understanding and replicating the physico-chemical interactions and deformation of protocell membranes under chemical signals will shed light on the fundamental mechanisms involved in the transition from pre-biotic compartments to cell-like architectures[9].***

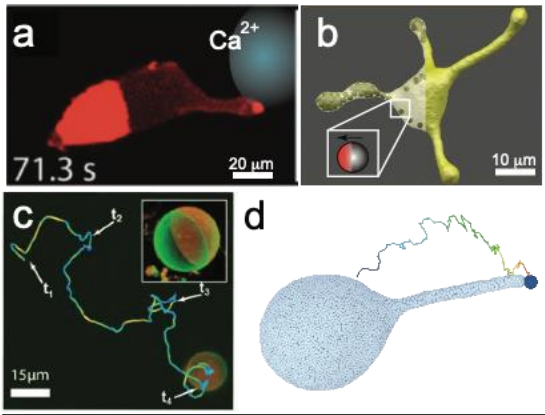


Fig.1 a) Artificial GUv deformation in the presence of Ca2+ gradients[14] b) anisotropic membrane deformation via active particles within a GUV in presence of H_2O_2 [15] c) motile Janus GUV with run and tumble dynamics[16] d) Numerical simulations by Munoz-Basagoti et. al. (unpublished work)

Artificial cell models, particularly Giant Unilamellar Vesicles (GUVs), offer a promising approach to mimic prebiotic protocell compartments. GUVs are synthetic analogues of cell membrane, typically formed by bilayers of amphiphilic lipids which allow for encapsulation of biomolecules and selective permeability. Their compartmentalized architecture allows communication[10,11] and interaction[12,13] with the environment. Motivated by chemically driven motility and membrane deformation, recent works have focused on engineering the induction of tubular protrusions through localized chemical stimuli with Ca^{2+} via spontaneous curvature[14], membrane-responsive GUVs, including the encapsulation of catalytic active colloids[15] and run-and-tumble motility of active phase separated GUVs due to their membrane fluidity properties[16] (***Fig.1a-c***), in contrast with simpler models using hard colloidal particles[17,18]. In particular, recent numerical simulations in the group of Prof. Šarić, showed the possibility of inducing deformation of lipid membranes mediated by external chemical cues (***Fig.1d***). These pioneering works underscore the potential of GUVs models as versatile platforms to investigate membrane rearrangement via chemical-driven signals between protocells. Nevertheless, up to date, there is not an out-of-equilibrium experimental system equipped with localized domain membrane deformation, motility and membrane-membrane interaction all driven by chemical signals. The combination of chemotactic active systems and GUVs might offer a model system to study these life-like behaviors. ***By leveraging minimal bottom-up systems, we can gain insights into the fundamental prebiotic interactions and the mechanical effects leading to membrane remodeling excluding complex biochemical process.***

2.2 Scientific Objectives

The aim of this proposal is to engineer a minimal artificial cell capable of chemically driven directed motion and membrane interactions via lipid membrane deformation. This is motivated by work in the group of our partner Prof. Šarić (ISTA), where numerical simulations point towards localized membrane deformation via chemically active membrane sites. The proposal will tackle the following objectives:

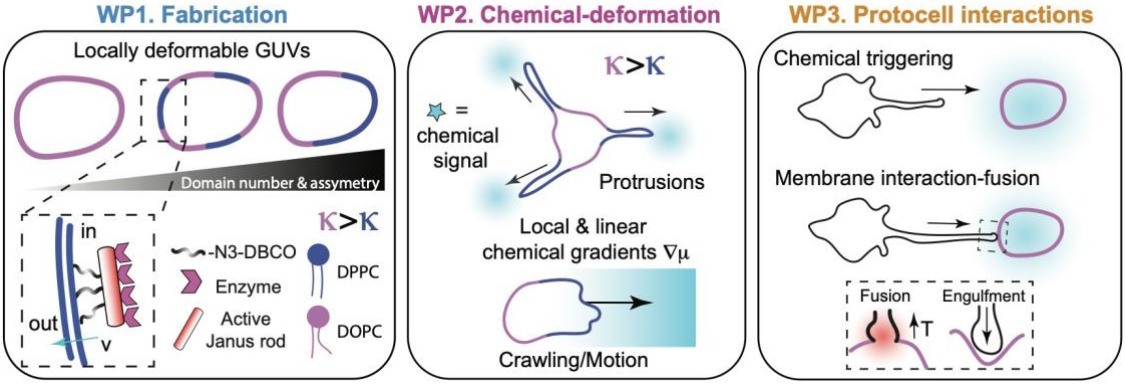
1. Fabricate a robust library of giant lipid vesicles with localized deformable lipid domains.
2. Study membrane deformations such as protrusion and GUV motion under controlled chemical fields.
3. Design chemically-driven protocell interactions via membrane deformation.

We will leverage the previous expertise of our group with bioinspired active matter, and phase-separated GUVS. We will closely collaborate with our partner in ISTA (Austria), Prof. Anđela Šarić, a specialist in equilibrium and out-of-equilibrium membrane modelling with Monte Carlo numerical models.

The outcome of the project tackles fundamental questions on membrane fluctuation and deformations in chemical landscapes of protocells, **bridging the gap between non-living and living matter.**

2.3 Methodology & Expected Results

This project is divided in three interconnected work packages, each of them tackling one objective.



WP1: Fabrication GUVs with localized deformable lipids domains *(6-8 months):* we will fabricate a library of giant lipid vesicles with chemically-deformable membrane domains, which upon interaction with external chemical gradients will drive localized membrane deformations. We will fabricate Janus GUVs (DOPC:DPPC:Chol)[19] with liquid- ordered (L_o) and liquid-disordered (L_d) domains, equipped with internal synthetic motors (enzymatic Janus rods). The fabrication of GUVs will be done with double emulsion technique (microfluidics)[20]. The two lipid domains will have asymmetric mechanical and adhesion properties, and will be experimentally designed based on the numerical observations of our partner at ISTA (Vienna). While the difference in bending modulus κ between both domains is intrinsic to their ordering state, adhesion to the surface will be tuned via external DNA binding[21]. The active particles will be used as enzymatically-driven motors which will be specifically attached to the deformable lipid domain (L_d, κ<10 kT) via click chemistry[22]. We will quantify membrane fluctuations, particle-membrane interactions and membrane stability. *This will result in the controlled bottom-up assembly of a novel design of multifunctional and asymmetric GUVs, with an extensive understanding of their physical-chemical and dynamical properties.*

WP2: Membrane deformation under chemical fields *(10 months, overlap with WP1):* Here, we will study membrane deformation under different chemical landscapes using flow-free microfluidic devices. We will investigate the ability of the vesicles to deform locally and self-propel due to the chemical interaction of its inner active units, probing the different types of enzymatic particles, membrane properties, and triggering molecules. The membrane deformation will arise from the swimming force exerted by the active rods pushing internally driven by enzymatic reactions (propulsion force f_p = 0.1 pN, Pe = $v_p r/D \approx 40$ to deform a membrane with bending moduli κ= 10 kT, estimated by Dr. Munoz-Basagoti (in agreement with Vutukuri et al.[15]). Depending on the lipid-domain spatial asymmetry, we will explore anisotropic deformation or directed motion. The membrane-surface interactions will be explored via DNA-binding to investigate the effect of local adhesion on membrane deformation. This behavior will be tested under controlled chemical gradients combined with obstacles to test the interaction and adaptation in complex environments (e.g., obstacles) included in microfluidic chips via soft-photolithography. *The outcome will deliver a chemically-responsive membrane with two deformation modes, leading to either anisotropic protrusion or crawling, inspired by chemotactic behavior of living organism. This will result in controlled membrane deformation of GUVs.*

WP3. Protocell membrane interactions *(12 months, after WP2):* The ultimate realization is the study of membrane interactions between two cell-like compartments. We will engineer two GUVs that communicate via long-rage chemical interactions. Briefly, the chemical signals of the first GUV -*producer* - will serve as triggering signal molecule of the second deformable GUV - *consumer*-, leading to local membrane protrusions of the latter that will extend until meet the *producer*. This chemical signaling will be explored using cascade -chain- enzymatic reactions between arginase[23] and urease[24], which have been never studied for membrane deformation. To allow chemical signals to diffusion through the membrane of the receiver, we will include membrane pore proteins (α-hemolysin)[25,26]. Once the membrane protrusion of the receiver meets the sender GUV, two scenarios will be explored: i) membrane fusion by increasing temperature of the sample through temperature chambers[27], and ii) penetration of the membrane protrusion on the receiver thanks to the swimming force of the inner motors. We will study the in-contact membrane-membrane fluctuations to extract mechanical information and lipid diffusion via FRAP. The experimental results will be supported by the numerical work on membrane-membrane interactions by Dr. Munoz-Basagoti using dynamically triangulated network models to simulate GUVs[28] in combination with non-reciprocal interactions as well as explicit chemical gradients to effectively account for environmental chemical cues. *This work package will deliver a minimal system to model first protocell interaction driven by chemical signals taking advantage of membrane deformations, proving information of the effect of self-directed protrusion in long-range protocell interactions.*

3. INTEGRATION IN FRONTIERS OF LIFE (1 PAGE MAX)

3.1 Alignment with the RRI objectives and integration in the network activities

ARCHEM present an innovative approach to engineer and study membrane motion and protocell-like interactions. This topic aligns with the Réseaux de Recherche Impulsion (RRI) and Frontiers of Life scheme's objectives constituting an innovative and interdisciplinary approach to a fundamental question in soft matter science: Can we go beyond the paradigm *'Only Cells Make Cells'*? By integrating principles from physics, chemistry, and biology, the project aims to understand, and mimic the minimal components for life-like behaviors. Collaboration with Prof. Anđela Šarić (ISTA) exemplifies the project's integration into a broader research network, fostering knowledge exchange and interdisciplinary cooperation. This research contributes to fundamental science by exploring the origins of life and the transition from non-living to living matter. The focus on constructing de novo-living systems offers potential insights into novel biomimetic materials and technologies. The outcome will be disseminated in high-impact journals and European and International Conferences.

3.2. Plan to fund research expenses

Main PI. Laura Alvarez Frances. The project costs and expenses will be supported by several existing fundings schemes. In particular, Assist. Prof Alvarez has been recently awarded the ARN-JCJC (202k euros) and ANR-PRCI-DFG (270k euros) to study the in equilibrium and out-of-equilibrium behavior of giant lipid vesicles (GUVs) and lipid monolayers, respectively. Assist. Prof. Alvarez currently holds a junior chair-pre recruitment (280k), supporting the purchase of various instruments (microscopes, cameras, microaspirations techniques). She is also a scientific partner (beneficiary of a DN-Marie Curie Doctoral Network on Synthetic Cells (SinSygCell, Coord: Prof. J.C. Baret), which will also support related expenses.

Scientific Partner. Prof. *Andela* Šarić: The group of Prof. Šarić has been already actively working on the project for the past two years, developing the idea and the theoretical proof of principle, supported by the MSCA ISTA "BRIDGE" Fellowship to Dr Maitane Muñoz-Basagoiti (~EUR 250k). These funds cover the salary, computational costs and consumables of Dr Muñoz-Basagoiti. In addition, Prof. Saric's ERC StG "NEPA" (~EUR 1.5 mil) and Vallee Fellowship (~$340k) support portions of this research related to archaeal reshaping.

3.3 Request

Requested resources		
Staff		
Duration (12 / 18 / 24 months)	*Supervisor*	*Hosting laboratory*
Postdoc 24 months	*Assist.Prof. ALVAREZ-FRANCES*	*Centre Recherche Paul Pascal (FR)*

3.4 Duration justification

The duration of the postdoc accounts for the intricate production process and the associated risks. ***The first 12 months*** are dedicated to optimizing and producing asymmetric lipid vesicles, involving composition optimization by V. Willems and microfluidic techniques. ***The second year*** focuses on symbiotic interactions and enzymatic cascade reaction optimization, extending the project's duration. All of the work will be closely guided and supported by numerical data from Prof. Šarić 's.

3.5 Long term perspective to continue the project

This collaborative project represents the beginning of a long-term collaboration with Prof. Šarić, dealing with out-of-equilibrium membranes, tackling fundamental questions of the origin of life. The success of the project will position Assist. Prof. Alvarez the front of the active matter community, with a novel and unique experimental out-of-equilibrium cell-mimetic system. Overall, support from FOL will support the growth of the PI's career and will help to increase her visibility amongst the French and international scientific communities, and increasing the chances for future funding acquisition. Prof. Šarić and Assist.Prof. Alvarez envision to apply for an ANR-PRCI and Austrian collaborative funds. Prof. Šarić and Dr. Munoz-Basagoti will be invited to participate as invited speaker to seminars at CRPP and in local Synthetic Cell workshops.

3. Consortium

OVERVIEW OF THE PARTNERS	
Partner 1	
Name of the PI	Assist. Prof. Laura ALVAREZ-FRANCES
Key personnel involved in the project	Postdoc recruited via FOL, PhD student (already recruited via Chair Junior, V.Willems) and postdoc ANR JCJC (starting in June 2024)
Role in the project	**Postdoc:** fabrication and characterization of asymmetric GUVs, characterization of mechanical properties and membrane deformations. Investigate the effect of chemical signals on membrane behavior. **PhD:** support with established knowledge in Phase-separated giant lipid vesicles
Organisation description Facilities involved in the project	The group of Alvarez is part of the Bio2.0 team at CRPP. Her lab is equipped with fluorescent microscopes, sample preparation lab, and recently acquire microsapirator to measure mechanical properties of GUVs. The CRPP has also a microfabrication facility for microfluidics, AFM, and confocal microscopy.
Partner 2	
Name of the PI	Prof. Andela SARIC
Key personnel involved in the project	**Postdoc :** Dr. Maitane Muñoz-Basagoitis
Role in the project	Numerical and theoretical support on the deformation of lipid membranes under chemical signals/gradients
Organisation description Facilities involved in the project	The group of Prof. Šarić is part of the Institute of Science and Technology Austria (ISTA). ISTA operates a large High-Performance Computing (HPC) computer cluster that allows for efficient large-scale numerical simulations (140 compute nodes and 20 GPU nodes (with at least 4 GPUs)
Team	
Five recent publications related to the project (published between 2020-2024) by the consortium members (not necessarily joint publications)	[1] Willems, V., Baron A.*, Matoz-Fernandez, D., Wolisfberg, G., Dufresne, E., **Alvarez, L***. Phase-separation dependent active motion of Janus Vesicles (2024, under review in *Nature Communications*). ArXiv: https://arxiv.org/abs/2311.00685 [2] Martin, N., **Alvarez, L*** et. al- Roadmap for Active and Animated Matter. Protocells section. *Journal of Physics: Condensed Matter* (2024 invited contribution – submitted). [3] Xie, K., Gorin, B., Cerbus, R. T., **Alvarez, L.**, Rampnoux, J. M, and Kellay, H. Activity induced rigidity of liquid droplets. Physical Review Letters, 129, 138001 (2022). [4] L. Harker-Kirschneck, A. E. Hafner, R. Henriques, B. Baum, **A. Šarić,** Physical mechanisms of ESCRT-III-driven cell division, Proc Natl Acad Sci U.S.A. 119, e2107763119 (2022). [5] C. Vanhille-Campos and **A. Šarić**, Dynamics of vesicle shape changes under osmotic shocks, Soft Matter 17, 3798 (2021).
Recent projects related to the current projects funded (between 2020-2024) by the consortium members (not necessarily joint projects)	**Assist. Prof. Alvarez**: - ANR JCJC (Chemical communication of active colloids 2022-2026 (270k EUR) - France Berkeley-Fund – Origami-driven active GUVs 2023-2024 (10K EUR) - Chair Junior IdEX 2022-2026 (280k EUR) - DN Marie Curie SinSygCell 2024-2028 Adaptive cell-mimetic systems under external actuation (Benefiary partner, 220K EUR) **Prof.** Šarić**:** -Valle Foundation Scholarship 09/2022 - 08/2026 ($340k) - ERC Starting Grant 10/2019 - 09/2024 “non-equilibrium protein assembly: from building blocks to biological machines” (1.5 mil EUR) - Volkswagen Stiftung Life? Grant 01/2020 - 12/2023 “The evolution of trafficking: from archaea to eukaryotes” (1.5 mil EUR, with 200k EUR as co-I) - Royal Society Research Grants for Research Fellows 10/2018 - 03/2024 (£97k) “Physical mechanisms of membrane remodelling by active elastic filaments” - Royal Society Research Fellows Enhancement Award 02/2018 - 03/2024 (£89k) “Rational design of cell-reshaping elements”
Pending joint proposal applications	**Not applicable**

Project title	Frontiers of Life
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Coordinator email address	Jean-christophe.baret@u-bordeaux.fr
Coordinator's lab	Univ. Bordeaux, CNRS, CRPP, UMR5031
Mots clés/Keywords	Structural Biology | Synthetic Biology | Regenerative Medicine | Molecular Machines | Supramolecular Chemistry | Soft matter | Biophysics | Organ-on-chips | Artificial cells | Minimal cells

Project team / Steering Committee		
Contact: rri.frontiersoflife.copil@diff.u-bordeaux.fr		
Name (lab)	Core competences	Role in Project Team
Jean-Christophe Baret (CRPP)	Microfluidics, Bottom-up Synthetic Biology, soft matter physics	Director. Representative of the project, in charge of project management
Hamid Kellay (LOMA)	Soft matter physics and biophysics	Physical aspects. In charge of training
Sébastien Lecommandoux (LCPO)	Soft matter chemistry, artificial cells and biomaterials	Bottom-up syn bio. In charge of innovation
Carole Lartigue (BFP)	Minimal cells and genetic engineering, Top-down synthetic biology	Top down syn bio. In charge of communication and outreach
Axel Innis (ARNA)	Structural biology, biochemistry, microbiology	Microbiology & structural biology
Joelle Amédée (BIOTIS)	Regenerative medicine, biomaterials	Biomaterials and medical applications. In charge of international relations.

Project PI		
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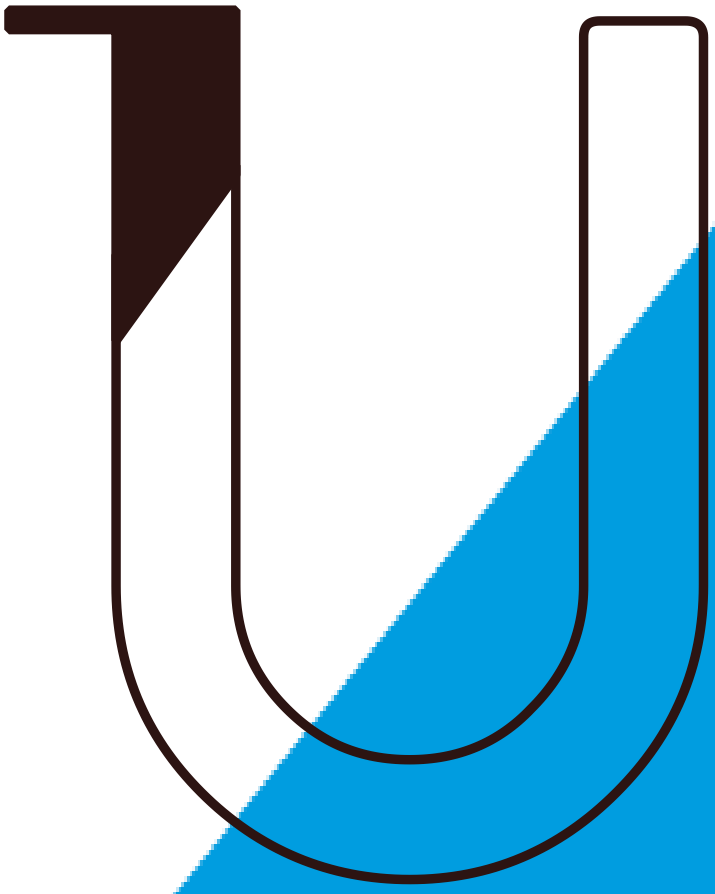

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