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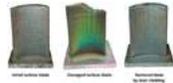
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LTC AENIGME

Laboratory for Trans-border Cooperation Aquitaine
Euskadi Network In Green Manufacturing and Eco-design

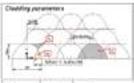
FRACTALS TO CHARACTERIZE THE MICROSTRUCTURE OF COMPONENTS REPAIRED BY ADDITIVE MANUFACTURING (LASER CLADDING)

Dr. Mario Alfredo Renderos Cartagena, Dr. Amaia Torregaray Laruscain, Prof. Nicolas Saintier, Prof. Eric Lacoste, Prof. Franck Andrés Girot Mata



MOTIVATION

Repairing worn metal parts using additive manufacturing (laser cladding), such as aircraft turbine blades, minimizes waste, energy consumption and environmental impact. In order to relate the component behaviour, it is important to relate the microstructure of the cladded part to the mechanical properties (static and fatigue) and to the cladding parameters.



PROBLEM

How to relate microstructure parameters such as grain size and aspect ratio, geometrical and crystallographic grain orientation, boundary network, dislocation density and residual stresses of each layer to the mechanical properties and process parameters (laser power, laser displacement speed, bead overlapping and offset, cladding strategy)?



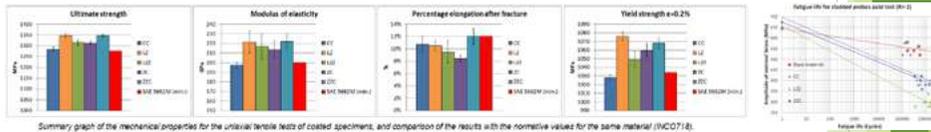
Example of self-similarity in grain morphology for an EBSD image of the CC deposition strategy on a coated specimen. The scale of the self-similarity will be limited in this case by the resolution of the image used in the fractal geometry analysis (the resolution in the EBSD image is 10 microns, the diameter of the cross-section shown is approximately 7.7 mm).

(a) Definition of the fractal dimension of the maximum random boundary connectivity (b) Demonstration of fractal analysis of MRBC by the box counting method.

SOLUTION: FRACTAL DIMENSIONS

The laser cladding process meets all the requirements to generate structures that can be characterized by fractal-like properties:

- > The material addition process will last as long as the laser beam supplies energy to the system (high energy in a given period of time).
- > Spatial targeting of the process (basically the entire system is concentrated in the molten bath area).
- > Non-equilibrium (the laser beam generates high temperature gradients that thermodynamically make the process move away from equilibrium).
- > Synergism is a reason that would explain why the microstructure generated in the laser supply process is different depending on the strategy used even using the same process parameters (the texture varies both in size and in the morphology of the grains).



Summary of the mechanical properties for the uniaxial tensile tests of coated specimens									
Fractal dimension	Class	Type	Resolution	UTS	YS	E	EP	EL	Mean
1.8	Non-cladded	Steel	1000	510.000	210.000	210.000	0.15000	0.15000	0.15000
1.8	Single-layer cladding	Steel	1000	510.000	210.000	210.000	0.15000	0.15000	0.15000
1.8	Double-layer cladding	Steel	1000	510.000	210.000	210.000	0.15000	0.15000	0.15000

Regression relations with central statistics for the variables of the ultimate tensile strength property from uniaxial tensile tests on cladded specimens									
Variable	Coefficient	Standard deviation	t-Student	Confidence Int.	Risk Int.	Control			
UTS	110.838	6.738	1.638	95.898	2.333	None			
YS	27.625	1.083	2.538	95.738	4.278	None			
E	1.55	0.002	7.808	99.998	0.002	None			
EP	-103.358	36.308	-2.838	99.938	0.078	None			

CONCLUSIONS

Fractal dimensions characterizing the microstructure are good indicators to describe the mechanical behavior of repaired components by laser cladding. The importance of the deposited layer was highlighted and in particular the parameters of the microstructure such as the grain size and the aspect ratio of the two layers, the crystallographic orientation of the grains of the intermediate layer which by epitaxy conditions those of the outer layer, and the geometric orientation of the grains of the outer layer on the UTS, YS, E and A% properties as well as the tension-compression fatigue limit.

ACKNOWLEDGEMENTS

This research work was carried out within the framework of the LTC AENIGME, thanks to the support of the LTC SAREA, the Basque Government and the Nouvelle Aquitaine Region.

Aenigme LTC

Fractals to Characterize the Microstructure of Components Repaired by Additive Manufacturing (laser cladding)

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INFRARED THERMOGRAPHY FOR THE QUALITY CONTROL OF SUSTAINABLE PROCESSES (LASER CLADDING AND MAGNETIC PULSE WELDING)

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MOTIVATION

Motivated by a societal imperative, this proposal addresses the urgent need for specialized inspection technologies to support the aeronautical and automotive industries' embrace of advanced manufacturing processes that align with society's demands for sustainability and efficiency.

On one hand, magnetic pulse welding, a groundbreaking technique that seamlessly joins aluminum alloys and steel, enables the introduction of lightweight aluminum alloys into vehicle production, contributing significantly to greenhouse gas reduction efforts.

On the other hand, the transformative power of additive manufacturing, particularly laser cladding, offers a sustainable alternative for repairing worn metal components, such as aircraft turbine blades. This technology minimizes waste generation, energy consumption, and environmental impact, aligning perfectly with the industry's commitment to environmental responsibility.

This proposal stands ready to fulfill the air and land transportation industries' aspirations for efficiency, sustainability, and safety, paving the way for a future where technological innovation meets societal needs.

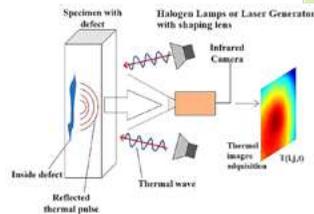


SOLUTION: INFRARED THERMOGRAPHY

Our proposed technological solution for inspecting these components is infrared thermography (IRT), a non-destructive testing technique known for its ability to perform safe, rapid, and contactless inspections of large areas. IRT works by inducing a thermal imbalance within the material and monitoring the subsequent changes in surface temperature using an infrared camera. Among the various excitation methods, optical stimulation with a homogeneous light beam stands out for its suitability in industrial-scale inspections. This technique combines a large excitation area with a wide detection field provided by the infrared camera, enabling the comprehensive inspection of sizable components.

Under homogeneous optical excitation, the absorbed radiation at the surface increases its temperature, initiating a heat flow that propagates into the material's depth. The thermal diffusivity of the material governs this heat flow, and defects disrupt this propagation pattern, resulting in variations in surface temperature. These temperature anomalies are effectively captured by the infrared camera.

IRT in this configuration serves two primary purposes that align with the project's objectives: firstly, it facilitates the depth-resolved measurement of thermal diffusivity, which is sensitive to the presence of apparent porosity in the material. Secondly, IRT excels at detecting buried defects parallel to the surface, such as poor adhesion in magnetic pulse welding (MPW) joints or porosities and lack of adhesion in additive manufacturing/laser cladding (AM/LC) processes.



GENERAL OBJECTIVES

- (1) Development of a non-destructive thermographic testing device for real-time monitoring of the quality of parts produced through sustainable manufacturing techniques: magnetic pulse welding (MPW) and additive manufacturing/laser cladding (AM/LC). This system will enable rapid and automated detection, characterization, and quantification of poor adhesion and porosity defects in metal components fabricated using these processes. Additionally, it will provide real-time identification of solidification microstructures (columnar or equiaxed) in AM/LC operations. The device's scalability will be demonstrated for industrial-scale implementation.
- (2) In-depth evaluation of the fatigue resistance of magnetic pulse welds and metal parts repaired or produced using AM/LC, considering the size and severity of defects present in the materials.
- (3) Identification of correlations between manufacturing parameters in MPW and AM/LC and the presence and significance of defects, leading to optimization of these parameters for enhanced part quality and reduced defect risk.

Project presented in the 2023 Call - "Knowledge Generation Projects" of the Ministry of Science, Innovation and Universities, Government of Spain. This research work will be carried out within the framework of LTC AENIGME, thanks to the support of LTC SAREA, the Basque Government and the Nouvelle Aquitaine Region.

Aenigme LTC

Infrared Thermography for the Quality Control of Sustainable Processes (laser cladding and magnetic pulse welding)

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 Amaia Torregaray,
 Jean Christophe Batsale
 Emmanuelle Abisset
 Alain Sommier
 Marie Marthe Groz

Introduction

Laser powder bed fusion (LPBF) is an emerging technology for the additive manufacturing of metal/composite products with complex shapes using powder materials. At the same time, the LPBF technology is a good tool to produce the Inconel superalloys with high precision, expanding manufacturing capabilities. Heat-resistance superalloys with favorable mechanical and physical properties are crucial materials for various relevant applications.

Despite the enormous potential of LPBF, this technology still has many challenges to use it for the manufacture of appropriate aircraft parts that work in extreme environments. The optimization of the LPBF parameters, powder reuse/recycling evaluation, and post-processing are required to achieve high-quality manufactured superalloy products for a critical manufacturing sector.

This work focused on the effects of reused powder on the porosity and surface texture of the LPBF-built IN 718 alloy parts. The reproducibility of thin-walled structures is also under consideration.

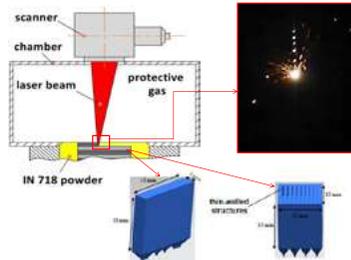


Fig. 1. Schematic illustration of the LPBF process

EXPERIMENTAL RESULTS

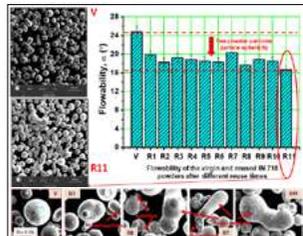


Fig. 2. Flowability in the LPBF-built IN 718 alloy after different reuse times

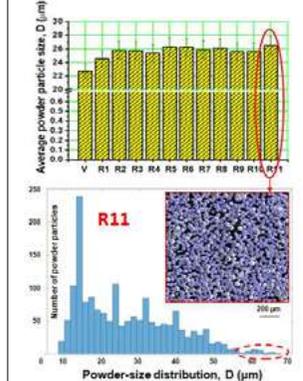


Fig. 3. Powder particle-size distribution in the LPBF-built IN 718 alloy after different reuse times



Fig. 4. Schematic representation of the powder reuse cycle: 1) LPBF process; 2) remove build plate; 3) sieve unmelted powder; 4) replace sieved powder into silo [1]

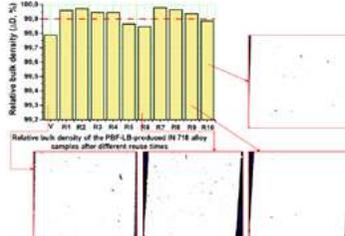


Fig. 5. Material density of the LPBF-produced IN 718 alloy parts after different reuse times

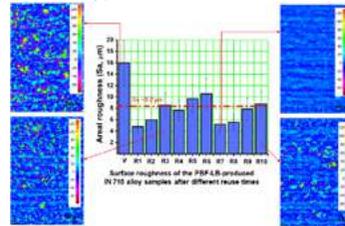


Fig. 6. Surface roughness and topography in the LPBF-produced IN 718 alloy parts after different reuse times

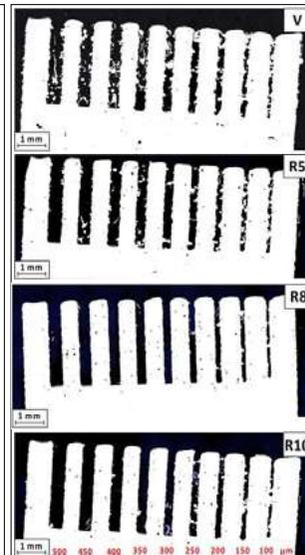


Fig. 7. Thin-walled structures in the LPBF-built IN 718 alloy parts using virgin powder (V) and reused powders (R) after the R5, R8, and R10 reuse times

REFERENCES

[1] https://issuu.com/inovar-communications/docs/magazine_metal_am_autumn_2017_app_-1177fr=sYm1MzEyMDA5MDQ

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The authors acknowledge the European Union's HORIZON 2020 research and innovation programme under the Marie Skłodowska-Curie Grant (agreement No. 101034379) and TED2021-130543B-I00 Grant funded by the MCIN/AEI/10.13039/501100011033 and the European Union Next Generation EU/PRTR.

Summary:

The powder reuse 10th iterations showed negligible effect on the material density (~99.9%) and surface roughness ($S_a \sim 8.2 \mu\text{m}$) of the LPBF-built IN 718 alloy parts.

Characterization of the reused powder showed a reduction in the number of smaller powder particles and an increasing number of irregular particles (powder particle size of irregular particles $L = 100\text{--}120 \mu\text{m}$, R6–R10).

LPBF-built thin-walled structures are characterized by better quality and repeatability using the reused powder.

Aenigme LTC

Laser powder bed fusion of Ni-based alloy components: Reused powder effects on surface/structural integrity and reproducibility of thin-walled structures

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Novel identification approach for quantifying and characterizing Abandoned, Lost, or Otherwise Discarded Fishing Gear (ALDFG)

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INTRODUCTION

Annual plastic pollution from abandoned fishing gear (FG) ranges from 28.4 kt to 100 kt, constituting 2.3% to 4.7% of global FG production [1]. To identify FG sources and complement existing marine litter classifications, we developed an easy-to-use identification-key (ID-K) (Fig. 2) that we applied on marine litter sampled in the southeastern Bay of Biscay (BoB) shores (Fig. 1).

M&M

- What? Sampling of beached marine litter
 - When? Seasonal sampling from Nov. 2022 to Nov. 2023
 - Where? 8 beaches of the southeastern BoB (Fig. 1)
 - How? OSPAR Marine Litter Monitoring Protocol
- Identification of the FG according to their fishing fleet (Fig. 2)
 - Polymer analysis through ATR-FTIR spectroscopy

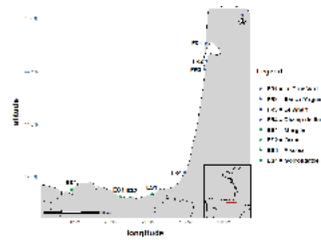


Fig. 1 : Studied area of the Plasfito research project and sampling beaches.

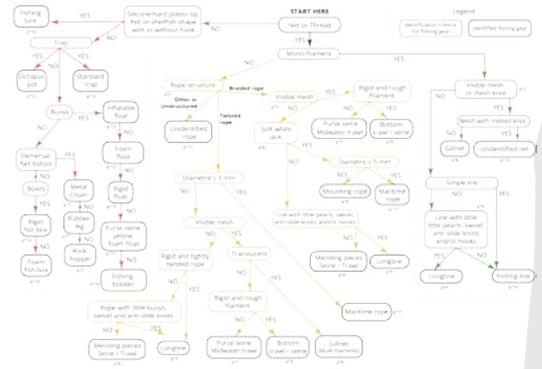


Fig. 2 : Identification key for abandoned, lost or otherwise discarded fishing gear.

RESULTS & ADVANTAGES OF THE NOVEL ID-K?

- Enable the identification of FG based on the fishing fleet they are used for and allow for the exclusion of items not related to the maritime sector.
- 14,462 items of plastic waste collected, 10.3% (1,486 items) of which came from the fishing and maritime sector.

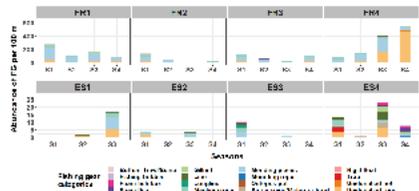


Fig. 3: Total amount of FG items collected by beach and by season. S1 = winter 2022-2023; S2 = Spring 2023; S3 = Summer 2023; S4 = Autumn 2023. E1-S4 not sampled due to bad weather.

- Most of the FG found are mending pieces (45.1%), unidentified ropes (37.6%) and maritime ropes (11.5%)
- Higher seasonal average of ALDFG on French sandy beaches (145±175 items/100m) than on rocky shores of the Basque Country (Spain) (6±7 items/100m)

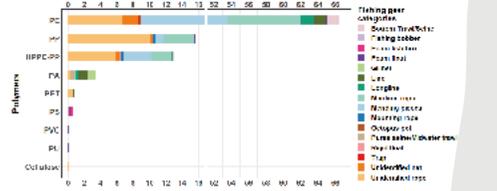


Fig. 4: Percentage of the polymer according to the fishing gear

- Predominance of ALDFG in PE, PP and HPPE-PP (polysteel).
- PA items mainly from gillnets and longlines, have rarely been found.
- Main hypothesis: the PA buoyancy is negative, and they are often lost with their weights. Thus a large part of lost FG is deposited on the seafloor and does not reach the shores.

CONCLUSIONS

- Mending pieces from repairing FG and their direct discarding into oceans or docks seem a relevant source of ALDFG. This type of ALDFG could be relatively easy to address.
- PA gear make up a large portion of lost FG, yet they rarely wash up on shores.
- Identifying the fisheries of origin is crucial for better managing plastic pollution from ALDFG.

FUNDINGS & PARTNERS:



AquEUS LTC

Novel Identification Approach for quantifying and characterizing Abandoned, Lost, or Otherwise Discarded Fishing Gear

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BACKGROUND

Plastics are a useful but persistent class of polymers. Environmental contamination by these plastics is a growing problem as they are known to cause harm to aquatic organisms by inhibiting the normal development and important cellular mechanisms whose effects vary by particle size and composition¹. Additionally, the production and use of volatile chemicals (VOCs) during polymer synthesis is a notable contamination risk. Biobased plastics and/or monomers have been suggested as a more degradable and renewably-sourced alternative for some of the most ubiquitous classes of polymers such as polyurethane, which is commonly used in various coatings, adhesives, sealants and foams. Moreover, the synthesis of waterborne polymers can reduce the production of volatile compounds.

OBJECTIVE & TASKS

- Assess the acute and sublethal toxicity of partially biobased waterborne polyurethane nanoparticle suspensions and their nanocomposites prepared with the incorporation of cellulose nanocrystals in freshwater zebrafish (*Danio rerio*) embryos and two zooplankton species: saltwater brine shrimp (*Artemia salina*) and freshwater rotifer (*Brachionus plicatilis*).
- In zebrafish embryos, acute toxicity was assessed by mortality during 120 hours of exposure and sublethal toxicity by the following parameters: malformation prevalence, hatching, and cell death analysis at 120 hours of exposure.
- In both zooplankton acute toxicity was assessed by mortality during 24 and 48 hours of exposure and sublethal toxicity by ingestion tests during 1 hour of exposure.

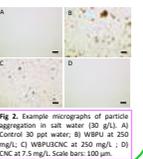
Partially-Biobased Waterborne Polyurethane Synthesis and Characterization

Nanoparticle suspensions synthesized from castor oil polyol, isophorone diisocyanate, and ethylenediamine chain extender.

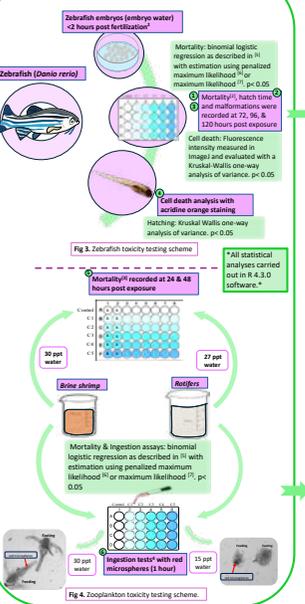
- WBPU: Partially biobased waterborne polyurethane
- WBPU3CNC: Partially biobased waterborne polyurethane nanocomposite (3% CNC)
- CNC: Bacteria synthesized cellulose nanocrystals

Fig 1. WBPU3CNC structure with WBPU and CNC components.

Exposure	WBPU (200 mg/L)	WBPU3CNC (200 mg/L)	CNC (6 mg/L)
Control (0h)	0	0	0
Exposure time (h)	17.9	17.9	17.9
Size (nm) (Median)	237.14	175.15	201.140
Size (nm) (Top)	175.15	201.140	175.15
Size (nm) (Bottom)	175.15	175.15	175.15



EXPERIMENTAL DESIGN



RESULTS

Fig 3. Zebrafish embryo A) survival rate (120 h) and B) mean hatching after waterborne exposure to different concentrations; $n=30$

Fig 4. Percentage of malformed embryos in some control groups, but lower in embryos exposed to the WBPU, WBPU3CNC and CNC. No indication of increased risk of specific malformations from exposure to the polyurethane suspension alone. Noticeable increase in malformation prevalence in embryos exposed to some concentrations of WBPU3CNC and CNC, specifically spinal & eye deformities and spinal deformity & edemas, respectively.

Treatment	Concentration (mg/L)	Total malformation rate (%)	Spinal deformity	Yolk-sac edema	Pericardial edema	Eye deformity
WBPU	0	17	0	0	0	17
	50	17	0	0	0	17
	100	17	0	0	0	17
	250	17	0	0	0	17
	500	17	0	0	0	17
	1000	17	0	0	0	17
WBPU3CNC	0	17	0	0	0	17
	50	13	7	0	0	7
	100	17	0	0	0	17
	250	17	0	0	0	17
	500	17	0	0	0	17
	1000	17	0	0	0	17
CNC	0	7	0	0	0	7
	1.5	7	0	0	0	7
	3	7	0	0	0	7
	7.5	7	0	0	0	7
	15	7	0	0	0	7
	30	7	0	0	0	7

CONCLUSIONS

- Polyurethane nanoparticle suspensions formed aggregates in saltwater media; this may have influenced zooplankton ingestion tests specifically.
- In zebrafish, the tested polyurethane suspensions and the nanocomposite reinforcement did not provoke acute toxicity, nor did they cause significant changes in embryo hatching or cell death.
- Elevated embryo malformation prevalence was observed after exposure to the polyurethane nanocomposite.
- None of the tested suspensions were acutely toxic to brine shrimps, but exposure to the polyurethane suspensions significantly increased mortality in rotifers at high doses (100 mg/L and 200 mg/L); the nanocomposite component alone also increased mortality significantly at 3 mg/L.
- The polyurethane suspension alone negatively impacted ingestion in both brine shrimps and rotifers. Exposure to nanocomposites caused a dose dependent decrease in brine shrimp ingestion. Alternatively, exposure to the highest concentration of the polyurethane nanocomposite suspension affected rotifer ingestion.
- This study highlights the importance of toxicity testing in different biological systems and with various sublethal parameters.

Zooplankton

Fig 5. Mortality of brine shrimp and rotifers at 24 and 48 hours of exposure to dilution series of (A) WBPU, (B) WBPU3CNC, and (C) CNC. Significant differences ($p < 0.05$) with respect to controls indicated by empty symbols; $n=30$.

Fig 6. Brine shrimp ingestion was negatively impacted; fasting increased along increasing concentrations of the WBPU and CNC; however, WBPU3CNC did not have a notable effect on ingestion. In rotifers, there was also a dose dependent increase in fasting when exposed to the WBPU. Interestingly, there was no major dose dependent increase in fasting when exposed to CNC but there was a significant increase in fasting when exposed to the highest concentration of the WBPU3CNC nanocomposite.

ACKNOWLEDGEMENTS

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AquEUS LTC

Toxicity assessment of partially biobased waterborne polyurethane nanoparticle suspensions in zooplankton and zebrafish embryos

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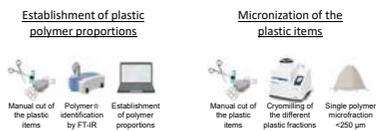
Introduction

Despite increasing micro and nanoplastic (MNPs) research, limited information on toxicity of realistic environmental MNPs (eMNPs) exists. This study aims to compare the toxicity to the rainbow trout liver cell line (RTL-W1) of leachates and DMSO and methanol extracts of micronized plastic items collected at different beaches of the Bay of Biscay.

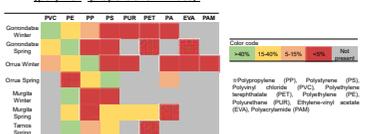
Materials and Methods



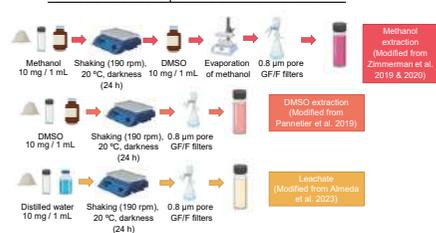
Plastics were collected on 3 beaches of the Spanish Basque Country (Gorrondatxe Orreaga and Murgita), in winter and spring 2023, and 1 beach in the French Basque Country (Tarnos) in spring 2021.



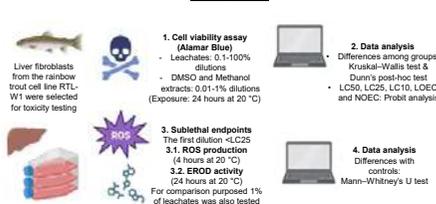
Obtention of micronized mix (polymer- proportions in mass):



Protocols selected to produce extracts and leachates



Cell exposure



*A minimum of 3 replicates of each treatment were used in all tests and tests were performed three times each.

Results

LC50, LC25, LC10, LOEC and NOEC after Alamar Blue cytotoxicity assay

Leachate	LC50	LC25	LC10	LOEC	NOEC
Gorrondatxe Winter	98,21 %	37,31 %	12,91 %	20%	10%
Gorrondatxe Spring	>100 %	24,85 %	4,78 %	20%	10%
Orreaga Winter	>100 %	35,41 %	8,83 %	20%	10%
Orreaga Spring	>100 %	36,04 %	10,84 %	20%	10%
Murgita Winter	>100 %	> 100 %	8,20 %	20%	10%
Murgita Spring	>100 %	98,72 %	20,27 %	40%	20%
Tarnos Spring	73,64 %	23,15 %	8,17 %	20%	10%

DMSO extract	LC50	LC25	LC10	LOEC	NOEC
Gorrondatxe Winter	X	X	X	0,1%	0,05%
Gorrondatxe Spring	X	X	X	0,01%	X
Orreaga Winter	X	X	X	0,05%	0,01%
Orreaga Spring	30,49%	4,29%	0,73%	0,5%	0,1%
Murgita Winter	X	X	X	X	1%
Murgita Spring	>100%	>100%	3%	0,05%	0,01%
Tarnos Spring	X	X	X	0,05%	0,01%

Methanol extract	LC50	LC25	LC10	LOEC	NOEC
Gorrondatxe Winter	X	X	X	0,1%	0,05%
Gorrondatxe Spring	X	X	X	0,01%	X
Orreaga Winter	X	X	X	0,01%	X
Orreaga Spring	>100%	>100%	6,57%	0,01%	X
Murgita Winter	X	X	X	0,05%	0,01%
Murgita Spring	>100%	>100%	0,16%	0,01%	X
Tarnos Spring	X	X	X	0,01%	X

ROS production:

	Leachate (20%)	Leachate (1%)	DMSO extract (1%)	Methanol extract (1%)
Gorrondatxe Winter	↑	↑	↑	↑
Gorrondatxe Spring	↑	↑	↑	-
Orreaga Winter	↑	↓	↑	-
Orreaga Spring	↑	↓	↑	-
Murgita Winter	↑	↑	↑	-
Murgita Spring	↑	↑	↑	-
Tarnos Spring	↑	↑	↑	-

↑: significant increase in ROS production in comparison to controls
 ↓: significant decrease in ROS production in comparison to controls
 -: no significant difference observed in comparison to controls
 Tested concentration: Leachates: 20% & 1%, DMSO extract: 1%, Methanol extract: 1%

EROD activity:

	Leachate (20%)	Leachate (1%)	DMSO extract (1%)	Methanol extract (1%)
Gorrondatxe Winter	-	-	↑	-
Gorrondatxe Spring	↑	↑	↑	↑
Orreaga Winter	-	↑	↑	↑
Orreaga Spring	-	↑	↑	-
Murgita Winter	↑	↑	↑	-
Murgita Spring	↑	↑	↑	↑
Tarnos Spring	↑	↑	↑	↑

↑: significant increase in EROD activity in comparison to controls
 -: no significant difference observed in comparison to controls
 Tested concentration: Leachates: 20% & 1%, DMSO extract: 1%, Methanol extract: 1%

Conclusions

- All leachates caused cytotoxicity at dilutions higher than 40% and almost all extracts at dilutions 51% on RTL-W1 cell line
- ROS production increased in cells exposed to all leachates at 20%, while different responses were observed for 1% dilution of leachates, DMSO and methanol extracts
- Similarly, EROD activity was induced in cells exposed to leachates and extracts of several tested samples
- Overall for leachates, Tarnos Spring was the most toxic mix. It showed the lowest LC50 and it increased ROS production and EROD activity even at 1% dilution. However, no clear trend was observed for DMSO and methanol extracts.
- These results highlight the complexity of analyzing toxicity of eMNPs due to differences in polymer types and proportions, additives, sorbed pollutants and aging degree

Acknowledgements

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AquEUS LTC

Toxicity to fish liver cell line of leachates and extracts from micro and nanoplastics produced from plastic items collected at different beaches of the Bay of Biscay

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Although Parkinson's disease (PD) is becoming increasingly important in ageing societies, its diagnosis still relies on the presence of classical motor symptoms that usually manifest in the later stages of the disease's progression. Patients may, however, present non-motor signs as cognitive and/or mood issues years prior to the onset of the motor ones. The Locus Coeruleus (LC) is one of the first areas to exhibit Lewy bodies and neurodegeneration. Its dysfunction is associated to the appearance of non-motor symptoms due to its noradrenergic (NA) projections and influence on the homeostasis of dopaminergic (DA) networks. We have set up a prodromal mouse model of human alpha-synuclein (α -Syn) overexpression at the LC by viral vector stereotaxic injections. Our goal is to characterize behavioural, functional, and structural changes driven by dysregulation of the LC-NA system, locally and in projecting areas such as the hippocampus or prefrontal cortex. Thus, we have studied behavioural phenotypes finding affectations related to spatial and working memories. Moreover, we have analysed structural changes in local inflammatory-related cells, as astrocytes and microglia, together with alterations in NA fiber network. These data, along with preliminary functional studies, provide an insight into how LC-NA dysregulation occurs at very early phases of PD and may aid in the search for early markers to facilitate its diagnosis.

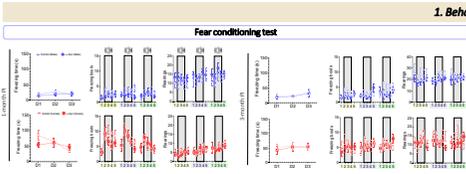


Fig. 1. Tone fear conditioning used to address aversive memory in α -Syn mice. The test was performed in 4 consecutive days: acquisition day (electrical shock + tone) and 3 test days (tone). No significant changes were seen between conditions in freezing and rearing parameters.

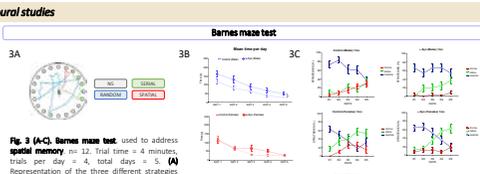


Fig. 3 (A-C). Barnes maze test, used to address spatial memory in α -Syn mice. Trial time = 4 minutes; trials per day = 4, total days = 5. (A) Representation of the three different strategies measured by the Barnes maze: NB, random, spatial. (B) Mean time to find the escape cage. α -Syn males trigger more time to find the escape cage, even if they present a normal curve of learning. (C) Percentage of strategies per day. As days go by, control males enhance their spatial strategy while α -Syn ones are not able to. The significant difference in this strategy suggests hippocampal affectations due to α -Syn.

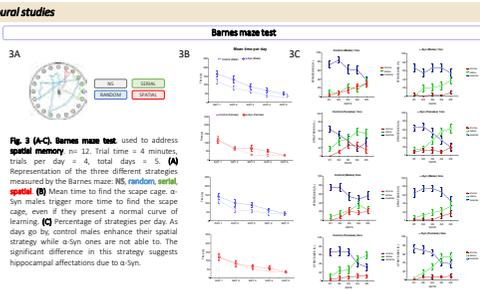


Fig. 2. Elevated Plus Maze used to address anxiety. n = 8. Trial time = 5 minutes. No significant differences were seen between conditions.

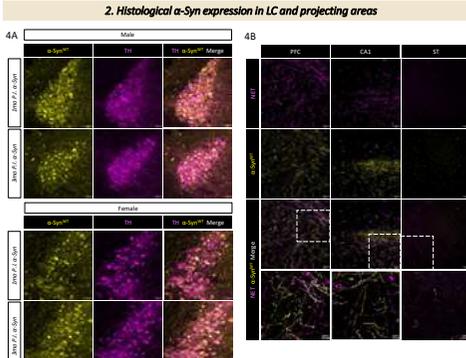


Fig. 4 (A) Immunofluorescence tile image of a unilateral injected mouse showing α -Syn^{WT} (green, left hemisphere) inside tyrosine hydroxylase (TH) neurons (red, merge in yellow). Scale bar = 30 μ m. **(B)** Immunofluorescence against Norepinephrine transporter (NET) (pink) and α -Syn^{WT} (yellow, merge in white). Confocal microscope images, 40x objective, immersion oil. Scale bar = 30 μ m, inserts' scale bar = 10 μ m.

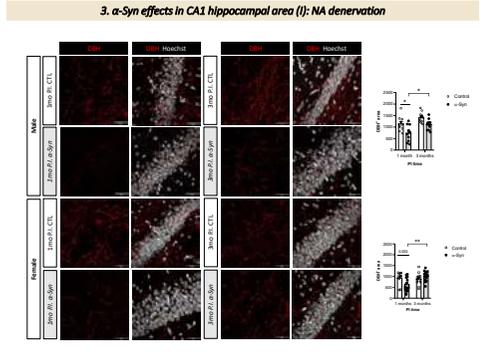


Fig. 5. Immunofluorescence against Dopamine beta hydroxylase (DBH) to check NA innervation in hippocampal LC-projecting area, CA1. Significant denervation is shown in 3-month PI, α -Syn mice than control homologues. Slight tendency to denervation is also seen in 3-month PI, α -Syn male mice. Confocal microscope images, 40x objective, immersion oil. Scale bar = 30 μ m. P values are represented by asterisks as follows: (*) p-value<0.05; (**) differences were considered significant when p-value<0.005.

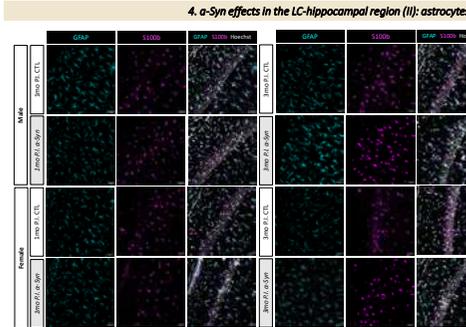
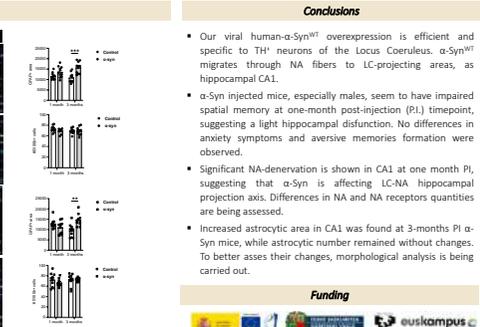


Fig. 6. To check astrocyte area (GFAP, blue) and number (S100b, magenta) in CA1 LC-projecting areas. Confocal IF images of CA1 astrocytes in male and female mice at 1 and 3-months PI. timepoints. GFAP+ area shows an increase in both male and female 3-months α -Syn groups. Number of astrocytes (S100b+ cells) is maintained through all analysed mice groups. Astrocytic complexity analysis is in progress. Scale bar = 30 μ m. Scale bar = 30 μ m. P values are represented by asterisks as follows: (*) p-value<0.05; (**) p-value<0.01; (***) p-value<0.001; (****) p-value<0.0001. Differences were considered significant when p-value<0.05.



Conclusions

- Our viral human- α -Syn^{WT} overexpression is efficient and specific to TH⁺ neurons of the Locus Coeruleus. α -Syn^{WT} migrates through NA fibers to LC-projecting areas, as hippocampal CA1.
- α -Syn injected mice, especially males, seem to have impaired spatial memory at one-month post-injection (PI) timepoint, suggesting a light hippocampal dysfunction. No differences in anxiety symptoms and aversive memories formation were observed.
- Significant NA-denervation is shown in CA1 at one month PI, suggesting that α -Syn is affecting LC-NA hippocampal projection axis. Differences in NA and NA receptors quantities are being assessed.
- Increased astrocytic area in CA1 was found at 3-months PI α -Syn mice, while astrocytic number remained without changes. To better assess their changes, morphological analysis is being carried out.



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Development of an Ecological Ultra High Performance Geopolymer Smart Concrete

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Project

The objective of NaturSea-PV is to improve the overall lifetime, reliability and maintainability of marine substructures for offshore floating photovoltaics (PV), to reduce degradation and failure rates, and thus investment risk and Levelized Cost of Electricity (LCOE).

Objectives

- Develop a Geopolymer UHPC and UHPC mix that serves as a benchmark.
- Test the designed G-UHPC to achieve the desired workability, compressive strength, and flexural strength.
- Conduct durability testing in both lab-scale conditions and in a harsh environment.
- Ensure the self-sensing (SHM) capability of the UHPCs enabling it to function as smart concrete.
- Perform a Life Cycle Assessment (LCA).

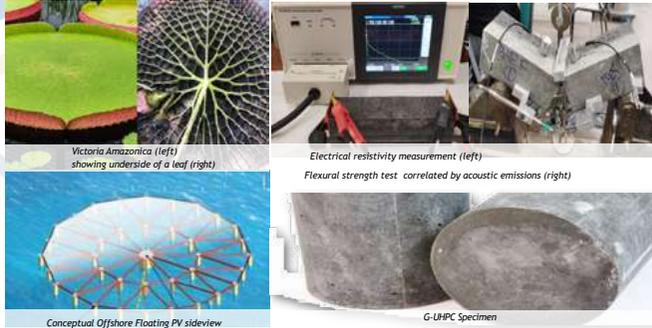
Our approach

NaturSea-PV will develop a lily-inspired PV substructure solution to meet the specific needs for Offshore Floating PV.

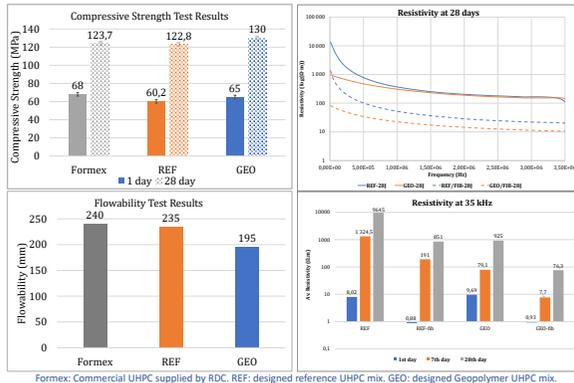
Ultra-high performance concrete (UHPC) will be coated with new biobased antifouling and anticorrosive coatings.

As part of the NaturSea-PV project, a new geopolymer UHPC is developed and optimized at I2M / University of Bordeaux.

Our team will support the computational tools team by validating experimental results within the modeling framework and we will lead the durability work package and contribute to other work packages.



Results



Conclusions

- G-UHPC has shown promising results when compared to traditional cement-based UHPC.
- UHPC mixes exhibited improved workability due to the inclusion of superplasticizers.
- Reference mix (REF) had higher resistivity values even within fibers included.
- The increase in resistivity values is attributed to the reduced water content in the specimens as they age over time.

Perspectives

- For evaluating the durability of UHPC mixes, a series of standardized tests will be conducted, including:
 - Chloride ingress testing according to ASTM C1556-04.
 - Sulphate, magnesium, and acid attacks will be conducted.
 - Salt attack will be tested in the harsh lab in Tecnalia/Spain & in the climatic chamber in the I2M lab.
- Large beam prototypes will be casted and tested on-site.

Partners



"Funded by the European Union. Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union or the European Climate Infrastructure and Environment Executive Agency (CINEA). Neither the European Union nor the granting authority can be held responsible for them."

Funded by the European Union

Green Concrete LTC

Development of Ultra High Performance Geopolymer Smart Concrete

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On the different statistics of Zero-Phonon-Line and Stokes-shifted photons emitted from interacting fluorescent quantum emitters

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CONSEJO SUPERIOR DE INVESTIGACIONES CIENTÍFICAS

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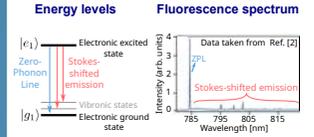
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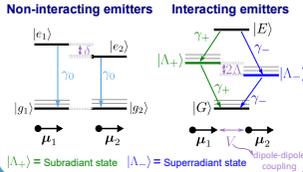
Motivation

Organic molecules and quantum dots have a very narrow Zero-Phonon Line (ZPL) at cryogenic temperatures [1], becoming promising candidates for quantum technologies.

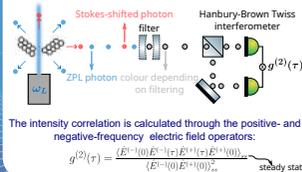


The relaxation from the electronic excited state gives raise to a photon emitted into the ZPL or to a Stokes-shifted photon.

The interaction between quantum emitters leads to the emergence of superradiant and subradiant states, having different lifetimes, energies and dipole moments [3,4].

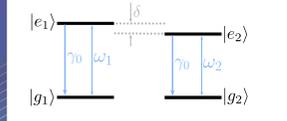


We analyze the intensity correlation of light emitted from two interacting emitters for different laser frequencies and intensities to better characterize this system.



Tailoring the correlation of ZPL photons [5]

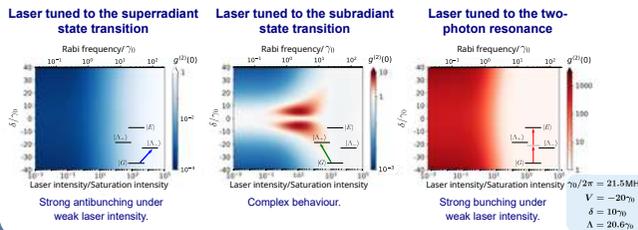
To characterize the properties of light emitted into the ZPL we use the typical microscopic description of the emitters, which ignores vibrational states and approximate them as two-level systems.



The intensity correlation is obtained using:

$$\hat{E}_{ZPL}^{(+)}(\tau) \propto |g_1\rangle\langle e_1|(\tau) + |g_2\rangle\langle e_2|(\tau)$$

The correlation of ZPL photons can be tailored in a wide range of values by tuning the laser intensity and/or frequency.



Addressing the correlation of Stokes-shifted photons

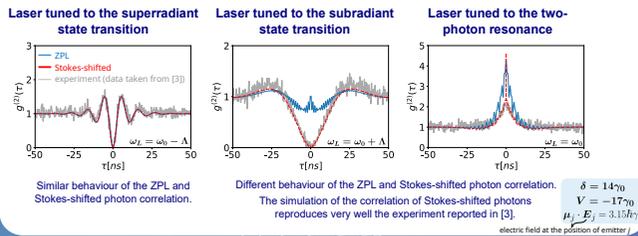
In available experiments [3,4], light is filtered and laser photons and ZPL photons are eliminated. Thus, only the properties of the Stokes-shifted photons are measured. To address such experiments, we propose a new microscopic model that includes additional 1-phonon states.



The correlation of Stokes-shifted photons can be obtained using:

$$\hat{E}_{St}^{(+)}(\tau) \propto |v_1\rangle\langle e_1|(\tau) + |v_2\rangle\langle e_2|(\tau)$$

The correlation of Stokes-shifted photons can show a very different behaviour than the correlation of ZPL photons.



Conclusions

- Interacting quantum emitters constitute a versatile photon source, whose statistical properties can be tailored with the laser intensity and frequency.
- The correlation of ZPL photons and of Stokes-shifted photons can be drastically different, which emphasizes the importance of using the appropriate microscopic model to describe each experimental configuration.
- Available experiments analyzing the correlation of Stokes-shifted photons are described well by including additional 1-phonon states in the microscopic description of the emitters and using the appropriate electric field operator.

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Translight LTC

On the different statistics of Zero-Phonon-Line and Stokes-shifted photons emitted from interacting fluorescent quantum emitters

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HYDROGEN INTERACTION WITH W(110) SURFACE



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ABSTRACT

An understanding of the dynamics of hydrogen atom scattering on metal surfaces is of great importance for a number of applications, including catalysis and material science. Of particular interest is the case of tungsten surfaces, as it will be the material employed for facing components in future fusion reactors. In this study, we investigate the scattering of hydrogen atoms on the W(110) surface, employing both classical and quantum dynamics approaches to elucidate the importance of quantum effects in this system. The focus of this study is on several key observables, including the sticking probability and diffraction channels, which are used to characterise the scattering process. Furthermore, the vibrational excitation spectra of H atom adsorbed on the tungsten surface is studied at both the semiclassical and quantum levels.

POTENTIAL ENERGY SURFACE

We make classical and quantum dynamics for hydrogen on W(110) using an accurate Potential energy surface (PES) calculated with PW91 functional.

$$V = V(x, y, z) \quad (1)$$

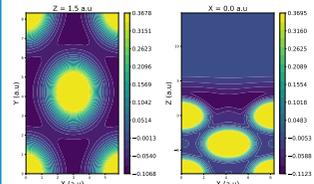


Fig. 1. Left (right) XY (XZ) cuts of the potential energy surface in the (2x1) cell). The hydrogen atom adsorption on hollow and bridge positions is energetically favorable.

MCTDH PARAMETERS

- Spatial grid with spacing $\Delta \approx 0.15$ a.u.
- The number of natural potentials chosen so that the fitting error < 1 meV
- We use fft SFPs basis with X and Y ones being periodic. The number of SFPs is chosen to converge sticking probabilities.

	X	Y	Z
Spatial-grid	41	55	155
Natpots	20	27	155
SFPs	24	32	64

Table: 1. Spatial grid, number of natural potentials (Natpots) used in the fit of the PES and number of SFPs per degree of freedom.

Initial Wave packet: We chose a product of plane waves for X and Y and a localized Gaussian in Z as the initial state. The initial position and momentum are x_0 and p_0 in normal incidence.

$$\psi_0 \sim e^{-ik_x x} e^{-ik_y y} e^{-((z-z_0)/2\sigma)^2} e^{-ip_z(z-z_0)} \quad (2)$$

STICKING PROBABILITY AFTER SCATTERING

- In the classical case, many trajectories are trapped in the surface at low energy, **trapping at the surface** has a shorter lifetime and is almost negligible after 1 ps when quantum dynamics are done.
- The latter differences are more pronounced at low energy resulting in a large quantum **reflection probability**.
- Bulk absorption** is enhanced in the quantum dynamical case due to tunneling effect, probably.

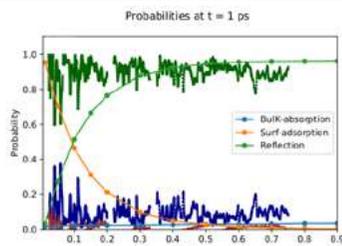


Fig. 2. Reflection (green), adsorption (red) and absorption (blue) probabilities of Hydrogen on W(110) under normal incidence conditions. Dark (light) lines correspond to quantum (classical calculations).

VIBRATIONAL SPECTRA

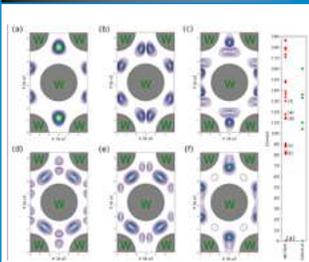


Fig. 3. Left: From (a) to (f) squared wave-functions for different vibrational states of Hydrogen adsorbed on W(110) surface. Right: vibrational spectra computed with MCTDH and a 1D quasiclassical approach.

DIFFRACTION

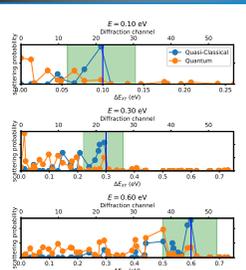


Fig. 4. Classical (blue) and quantum (orange) diffraction probabilities for H scattering from W(110) with normal incidence as a function of energy transfer to transversal directions.

ONGOING WORK

It is known that electronic friction plays a crucial role for atoms scattering on metals. We plan to use the stochastic version of MCTD to include this energy dissipation channels

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QuantumChemPhys LTC

Hydrogen Interaction with W(110) Surface

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BioMolecular Interactions Platform

Cross-border Cooperation Laboratory Network San Sebastian - Bordeaux

BIOMINT

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Molecular Biophysics, (Bio)Organic Chemistry, Sensor Technology, Materials Science, (Bio)Chemical Engineering



Our Motivation

Biomolecular Interactions are at the core of designing and employing advanced and functional materials in biotechnology, bionanotechnology, health, and biosensors as well as separation and purification processes in, for example, fine chemicals and pharmaceutical manufacturing. Yet, their measurement and characterization often pose a challenge.

What is BIOMINT about?

- Trans-border technological platform that offers its expertise on biomolecular interactions to both the scientific community and industry in the area of **bio/materials/health**.
- Vibrant collaboration network in both applied and fundamental research, promoting best laboratory practices when it comes to determining biomolecular interactions.

How BIOMINT works

Example: Insights into binding mechanisms for the design of sensors for the detection of SARS-CoV-2

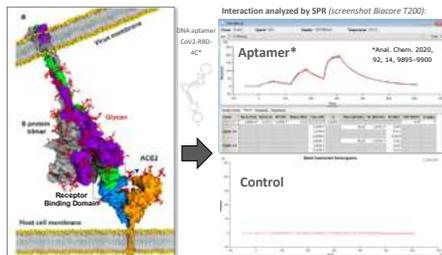


Image: Taka et al. 2021, J Phys Chem.

Aptamers can rival antibodies for recognizing the RBD of the spike protein. Within an international consortium, we have observed that some supposed RBD-aptamers actually do not interact specifically and that some literature data lack rigour or are based on measuring artefacts. This demonstrates how crucial it is to investigate molecular interactions with effective and straightforward methods prior to potential health applications.

Additional support from...

Institutions:



Private companies:



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BioMINT LTC incubator

BioMolecular Interactions Platform

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Available Technologies



The use of complementary techniques warrants robustness of the interaction data obtained and avoids interpreting artefacts. During our varied research activity we have learnt that thermodynamic constants of biomolecular interactions not only vary with the characterization technique but also depend on the particular application and environment.

BIOMINT Activities

- Hands-on workshop (Univ. Bordeaux annual summer school) on the use of surface-sensitive techniques for elucidating biomolecular interaction on surfaces
- Training of young researchers within collaborative projects
- Seminar series between Bordeaux and San Sebastián
- Joint projects in the bio/materials and health interface

What is in for me?

- BIOMINT is an **open platform** that welcomes partners from **both academia and industry**.
- Access to expertise on characterizing biomolecular/materials interactions with a set of complementary techniques that are not widely accessible.
- Unique opportunity for **young researchers** to be trained in state-of-the-art characterization techniques.

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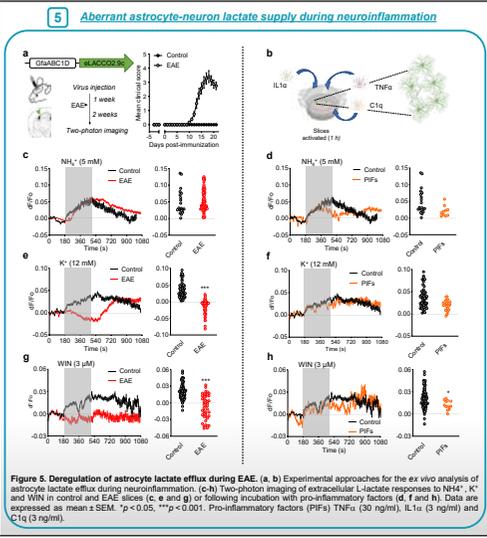
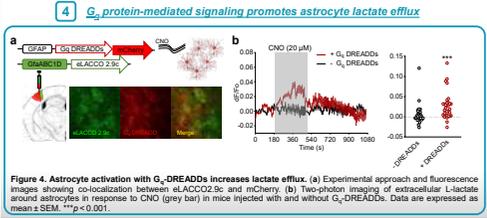
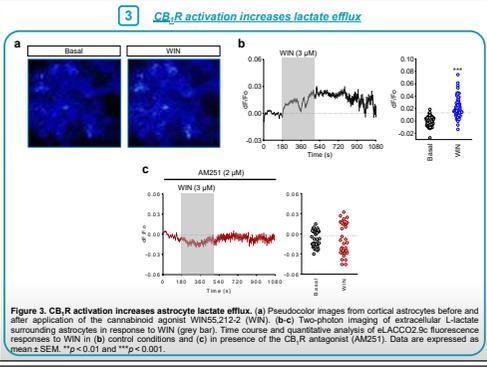
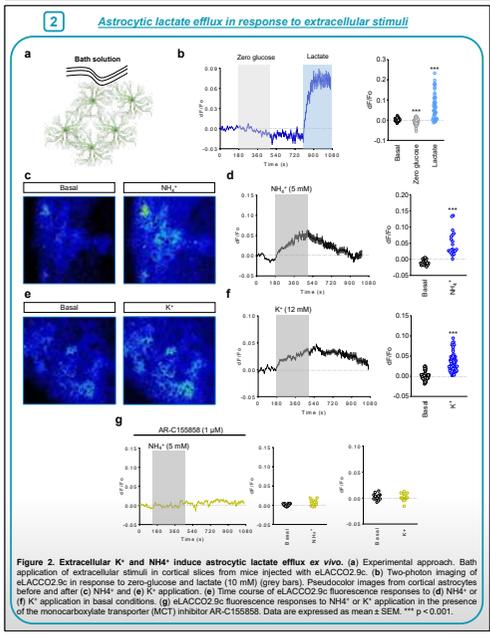
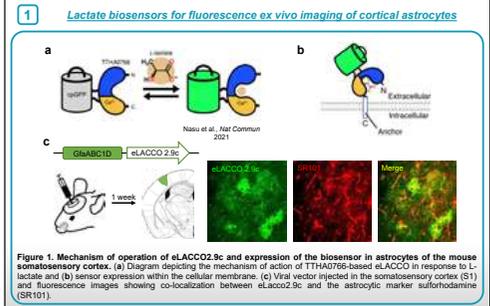
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Introduction

Astrocytes are primarily glycolytic cells that produce and release lactate to support neuronal aerobic metabolism during neurotransmission. This so-called astrocyte-neuron lactate shuttle is tightly regulated by neuronal signals that trigger aerobic glycolysis in astrocytes and regarded as an essential mechanism underlying astrocytic modulation of brain performance.

Multiple sclerosis (MS) is a chronic demyelinating disease initiated by pathogenic immune responses against myelin followed by a broader inflammatory and neurodegenerative process. Astrocytes undergo a pronounced transformation in MS whereby they acquire a variety of disease-promoting functions. In particular, accumulating evidence suggests that deficits in astrocyte glycolytic activity encompass the pathogenic activation of these cells during neuroinflammation and contribute to neurodegenerative processes. However, the specific features of astrocytic lactate supply to neurons in multiple sclerosis (MS) remain unknown.

Here we used newly generated, genetically coded lactate biosensors to investigate **astrocyte lactate efflux** induced by physiological stimuli associated with neuronal activity. *Ex vivo* two-photon imaging of extracellular lactate using eLACCO2.9 targeted to astrocytes of the mouse somatosensory cortex showed positive responses to extracellular K⁺, NH₄⁺ and to thus confirming previous observations in culture systems. Cortical astrocytes also displayed eLACCO2.9 fluorescence increases in response to the cannabinoid agonist WIN5,521-2 that were prevented by the selective CB₁ antagonist AM251. Our preliminary results in cortical slices suggest decreased lactate efflux induced by extracellular WIN5,521-2 in cells activated with pro-inflammatory stimuli (IL-1 α , TNF α and C1q). *Ex vivo* astrocyte lactate imaging in the experimental autoimmune encephalomyelitis (EAE) model of MS showed a decreased lactate efflux induced by K⁺ and CB₁R activation. These observations support the utility of eLACCO2.9c as novel imaging tool to explore the features of astrocyte-neuron lactate supply *ex vivo* and suggest aberrant astrocyte-neuron communication in terms of lactate supply during neuroinflammation.



Summary

- Viral targeting of cortical astrocytes with eLACCO2.9c allows real-time measurement of extracellular lactate fluctuations *ex vivo*.
- Cortical astrocytes display positive eLACCO2.9c fluorescence responses to extracellular K⁺, NH₄⁺ and CB₁R activation that are sensitive to the pharmacological blockade of lactate transport.
- Activation of G_q protein-mediated signaling promotes astrocyte lactate efflux in the mouse cortex.
- Inflammatory activation of cortical astrocytes *ex vivo* and *in vivo* deregulates lactate supply.

Acknowledgements
 This study was supported by Instituto de Salud Carlos III (ISCIII) through the project (PI21/00629) and co-funded by the European Union, the Basque Government (PIBA_2023_1_0046) and ARSEP Foundation (ARSEP-1310).

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Imaging astrocyte-neuron lactate supply in multiple sclerosis

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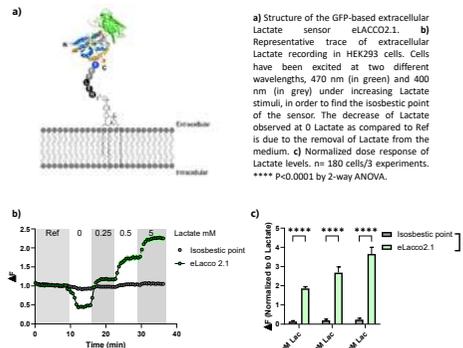
In vivo imaging of CB1-dependent modulation of brain metabolism

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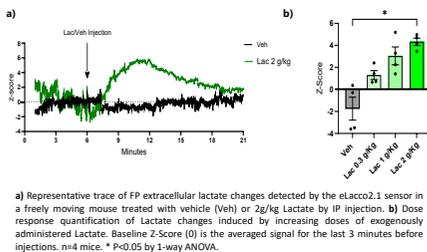
INTRODUCTION

Despite representing only the 2% of the body weight, the brain consumes up to 20% of the body energy. Most of the brain energy comes from the full oxidation of glucose. However, a sizeable amount of glucose is partially oxidized and transformed into Lactate via a process called aerobic glycolysis. Most current data point indicate that astrocytes are the main producers of Lactate, which is in turn used by neurons to fulfill their energy requirements. Interestingly, Lactate does not only play a metabolic role, but it is also a fundamental brain signaling molecule. The mechanisms regulating Lactate production and release in the brain are, however, scantily known. Cannabinoid receptors type 1 (CB1R) has recently emerged as an important factor playing a complex role in these processes. Whereas persistent (24h) activation of astroglial CB1R leads to a reduction of Lactate¹, our recent data show that their transient stimulation (5 min) triggers an increase of Lactate levels². These data, however, were obtained mainly in cultured astrocytes, and the impact of CB1R activity on Lactate levels in vivo has never been directly measured. By combining a novel Lactate fluorescent biosensor (eLacco2.1)³ and fiber photometry (FP), I observed that the levels of Lactate correlate with locomotor activity and that Lactate administration blocks hypolocomotor effects of cannabinoids. Current experiments aim at addressing the potential mechanistic link between locomotor effects of cannabinoids and Lactate levels regulation.

1) CHARACTERIZATION OF eLACCO2.1



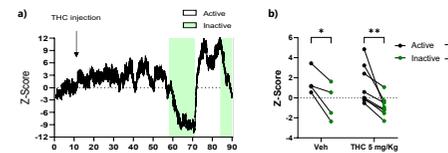
2) IN VIVO LACTATE RECORDINGS



PARTENAIRES

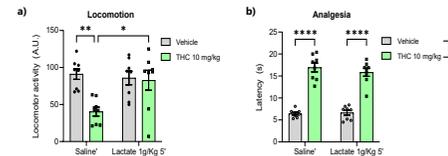


3) IMPACT OF LACTATE LEVELS ON LOCOMOTION



The hypolocomotor effect of THC is correlated with a reduction of Lactate levels in the brain. **a)** Representative trace of FP extracellular Lactate changes detected by the eLacco2.1 sensor in a freely moving mouse. Arrow indicates the IP injection of 5 mg/kg THC. Green areas indicate inactive behavioral phase. Recordings lasted 90 minutes, with injection after 11 minutes. **b)** Quantification of Lactate changes during the shift from active to inactive phases under vehicle or 5 mg/kg THC. n= 8 mice. 2-Way Anova. **, P<0.01 (General effect of activity).

4) ROLE OF LACTATE IN LOCOMOTOR EFFECTS OF CANNABINOIDS



The hypolocomotor, but not antinociceptive, effect of THC is blocked by Lactate injection. **a)** Locomotor activity (activity cages) and **b)** Thermal antinociception (hot plate) of mice treated with vehicle or THC 10 mg/kg, followed by saline or Lactate 1 g/kg 5 min after. 2-way ANOVA: **a)** Interaction (p=0.0175); * P<0.05, ** P<0.01; **b)** Main effect of THC, **** P<0.0001.

CONCLUSIONS AND FUTURE DIRECTIONS

In the first part of this PhD project we effectively proved that:

- The isobestic point of eLacco2.1 is at 400 nm (fundamental for fiber photometry recordings).
- It is possible to measure lactate changes in freely moving mice with fiber photometry.
- The best lactate dose in order to observe a lactate increase without saturating the sensor is 2g/Kg.
- The hypolocomotor effect of THC is correlated with a reduction of Lactate levels in the brain.
- The hypolocomotor, but not antinociceptive, effect of THC is blocked by Lactate injection.

In the future, we are planning to:

- Analyze different behaviors related to Lactate changes during locomotion (DeepOF).
- Explore CB1-dependent modulation of Lactate on locomotion.
- Study the impact of locomotion on brain Lactate levels in basal ganglia (Striatum, Substantia nigra and globus pallidus).
- Confirming the blocking effect of Lactate on THC-induced hypolocomotion by local injections into these brain regions, and by intracerebroventricular injections.
- Explore the cellular and molecular mechanisms of the relationship between cannabinoids, Lactate and locomotion.

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In vivo imaging of CB1-dependent modulation of brain metabolism

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ABSTRACT
Oligodendrocytes (OLs) produce central nervous system myelin thus providing protection and metabolic support to axons. Myelin formation and repair are energetically expensive and oligodendrocyte metabolic defects have been postulated as a cause of neurodegeneration. Endocannabinoids modulate neuroglial metabolism through the activation of CB₁ receptors (CB₁Rs). Myelinating OLs and precursor cells (OPCs) express CB₁Rs promote lineage progression but the metabolic implications of these effects in health and disease remains uncertain. Here we investigated the energy metabolism of oligodendroglial lineage and the role of CB₁Rs in oligodendrocyte energy metabolism *in vitro*.
Seahorse analysis revealed that OLs exhibited a higher glycolysis metabolism compared to OPCs. Additionally, lactate extracellular biosensor eLACCO2.9 revealed differences in lactate release between OLs and OPCs. Seahorse analysis showed inhibitory effects of the CB₁R agonist ACEA on mitochondrial respiration in OLs cultures. The antagonist AM251 prevented the inhibitory effect of ACEA on maximal OCR. These results suggest that oligodendrocyte differentiation is associated with changes in energy metabolism *in vitro* and CB₁Rs modulate mitochondrial respiration in OLs, possibly mediated by mitochondrial CB₁R.

Methods: Oligodendrocyte cultures

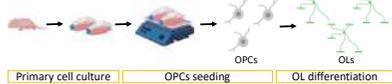


Figure 1. Experimental procedure for oligodendrocyte cultures. Cells were isolated from the cortex of postnatal rats and maintained as mixed glial cell cultures for 10-15 days. OPCs were plated and maintained as OPCs or differentiated to OLs.

Mature oligodendrocytes are more glycolytic than OPCs *in vitro*

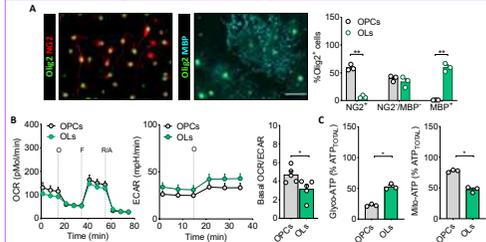


Figure 2. Mature oligodendrocytes show a higher glycolytic rate than OPCs. (A) Immunofluorescence images of OPC-enriched cultures (left) and OLs cultures (right). (B) Mitostress Seahorse analysis of oxygen consumption rate (OCR), extracellular acidification rate (ECAR) and ratio between basal OCR and basal ECAR. (C) ATP rate Seahorse analysis of ATP produced from glycolysis or mitochondrial respiration. **p* < 0.05; ***p* < 0.01. Student's *t*-test. Scale bar: 50 μm.

Lactate biosensor for fluorescence *in vitro* imaging of oligodendroglia

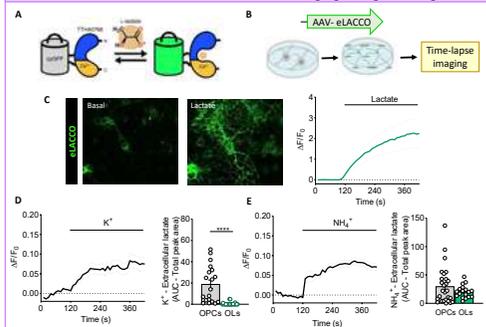


Figure 3. Extracellular K⁺ and NH₄⁺ induce oligodendroglial lactate efflux *in vitro*. (A-B) Diagram of the mechanism of action of eLACCO in response to L-lactate and expression in oligodendroglial cells. (C) eLACCO fluorescence response to lactate (10 mM). (D-E) Time course of eLACCO fluorescence responses to K⁺ and NH₄⁺ in OPCs and OLs. ****p* < 0.001. Student's *t*-test. Scale bar: 50 μm.

CB₁Rs modulate mitochondrial metabolism in oligodendrocytes

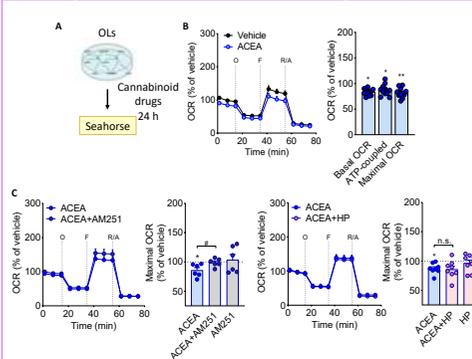


Figure 4. Agonist activation of CB₁Rs inhibits mitochondrial energy metabolism in oligodendrocyte cultures. (A) Diagram of OLs treatment. (B) ACEA (25 nM) reduced OCR in OLs. (C) AM251 treatment blocked maximal inhibition of OCR triggered by ACEA in OLs. (C) Hemipressin (HP; 25 nM) did not block maximal inhibition of OCR triggered by ACEA. **p* < 0.05; ***p* < 0.01; ****p* < 0.001; *****p* < 0.0001 Student's *t*-test and One-way ANOVA.

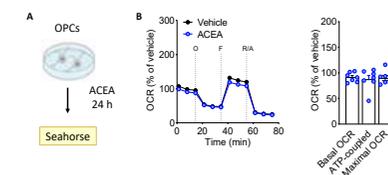


Figure 5. CB₁R does not modulate mitochondrial metabolism in OPCs. (A) Diagram of OPCs treatment with ACEA (25 nM). (B) ACEA did not modulate OCR in OPCs. Student's *t*-test.

CB₁Rs do not modulate glycolysis in oligodendrocytes

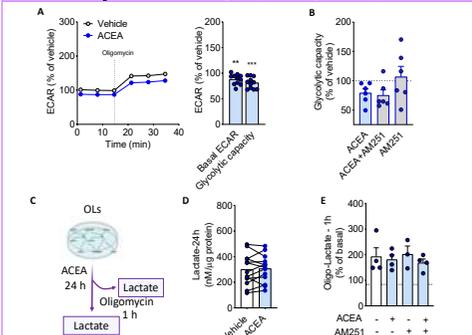


Figure 6. Agonist activation of CB₁Rs does not modulate glycolysis in oligodendrocytes. Treatment with ACEA (25 nM) reduces ECAR in OLs but AM251 (B) did not block this effect. (C) Diagram of the experimental procedure for extracellular lactate assay. (D) Extracellular lactate released after 1 hour by cells treated with ACEA during 24 hours. (E) Measurement of extracellular lactate under 1 hour oligomycin treatment. **p* < 0.05; ***p* < 0.01. Student's *t*-test.

CONCLUSIONS

- OPC differentiation involves the modulation of bioenergetic profiles.
- CB₁R agonist inhibits mitochondrial respiration in oligodendrocytes via mitochondrial CB₁R.
- Oligodendrocyte CB₁Rs do not modulate glycolysis.

Acknowledgements

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CannaMetHD LTC incubator

Oligodendrocytes energetic metabolism: modulation by CB1 receptors

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CannaMetHD LTC incubator

Specialized cannabinergic astrocytes may mediate eCB activity in mice

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INTRODUCTION

The endocannabinoid (eCB) system is a widely distributed, polyfunctional signaling system that is virtually involved in all brain functions. In the brain, eCB production has been always attributed to neurons, in a process involving postsynaptic depolarization and calcium increases. However, astrocytes express all the machinery to produce eCBs, although their role in eCB production and signaling has never been studied in detail due to the lack of suitable techniques to detect the source of eCB in real time. In this project, we took advantage of the novel eCB sensor GRABeCB2.0, a fluorescent biosensor consisting of a human CB2 receptor (CB2R) coupled to a GFP protein which allows to detect eCB in real time. Expressing the sensor in astrocytes *in vitro* and *ex vivo*, we were able to study the dynamics of eCB production and release in astrocytes, which show some interesting features that may have important consequences in astrocyte-neuron communication and eCB signaling.

RESULTS

1) VALIDATION OF THE SENSOR

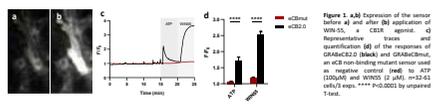


Figure 1. a) Schematic of the sensor before and after application of WIN55,212-2, a CB2R agonist. b) Representative traces of the response of GRABeCB2.0 (black) and GRABeCB2.0-Delta (grey), an eCB non-binding mutant sensor used as a negative control, to ATP (100µM) and WIN55 (2 µM). n=22-61 cells/3 exp. *** P<0.001 by unpaired T-test.

7) EX VIVO ASTROCYTE ACTIVATION

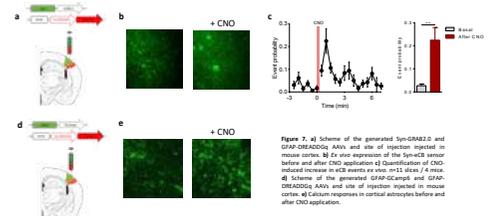


Figure 7. a) Schematic of the generated Spn-GRABeCB2.0 and GFAP-DREADD2sCre (AAV) and site of injection. b) Ex vivo expression of the Spn-GRABeCB2.0 sensor before and after CNO application. c) Quantification of CNO-induced events in ex vivo astrocytes. n=11 slices/4 mice. d) Schematic of the generated GFAP-Cre and GFAP-DREADD2sCre AAV and site of injection. e) Ex vivo expression in cortical astrocytes before and after CNO application.

2) SPONTANEOUS eCB EVENTS

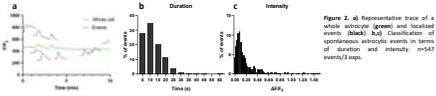


Figure 2. a) Representative trace of a spontaneous event. b) Classification of spontaneous events in terms of duration and intensity. n=547 events/3 exp.

3) IDENTITY OF eCB

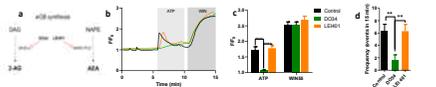


Figure 3. a) Schematic of the two eCB synthesis pathways, with their respective inhibitors: DCGI4 for 2-AG and URB594 for anandamide (AEA). b) Representative trace of the response to ATP and WIN of astrocytes pretreated with DCGI4 (green) or URB594 (orange) and respective quantification. n=47-67 cells/2 exp. *** P<0.001 by one-way Anova test. c) Quantification of the frequency of spontaneous events in astrocytes treated with DCGI4 (green) and control (black). n=12-18 astro/2 exp. ** P<0.01 by unpaired T-test.

8) TRANSCRIPTOMIC ANALYSIS OF DAGLA-POSITIVE ASTROCYTES

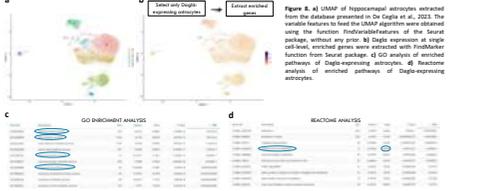


Figure 8. a) Schematic of hippocampal astrocytes extracted from the database generated in the Dagle et al., 2012. The variable features to feed the SIMAP algorithm were obtained using the function 'findPathways' of the Simap package, without any prior. b) Dagle expression in single cell level, enriched genes were extracted with the cluster function from Seurat package. c) GO analysis of enriched pathways of Dagle-expressing astrocytes. d) Reactome analysis of enriched pathways of Dagle-expressing astrocytes.

4) Ca²⁺ eCB DYNAMICS

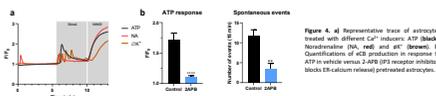


Figure 4. a) Representative trace of astrocytes treated with different Ca²⁺ indicators: ATP (black), Nipradistal (DA, red) and aR²⁺ (brown). b) Quantification of eCB production in ATP vs vehicle versus 2-APB D2R receptor inhibitor, blocks calcium released from astrocytes.

9) PATHWAY FOR ASTROCYTE-SPECIFIC eCB SYNTHESIS

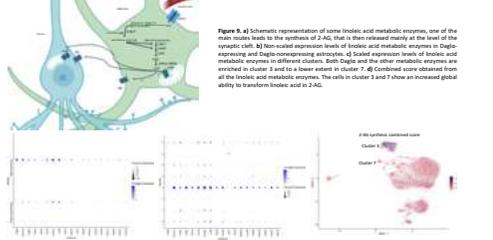


Figure 9. a) Schematic representation of some lipase and metabolic enzymes, one of the most enriched by the analysis of DAGs. b) Heatmap of enriched pathways of DAGs. c) Heatmap of enriched pathways of DAGs. d) Heatmap of enriched pathways of DAGs.

5) ASTROGLIAL eCB RELEASE

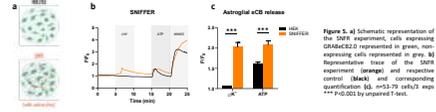


Figure 5. a) Schematic of the generated Spn-GRABeCB2.0 and site of injection. b) Representative trace of the SNFRP response (green) and respective control (black) and corresponding quantification. n=13-17 cells/2 exp. *** P<0.001 by unpaired T-test.

6) EX VIVO ASTROGLIAL eCB DYNAMICS

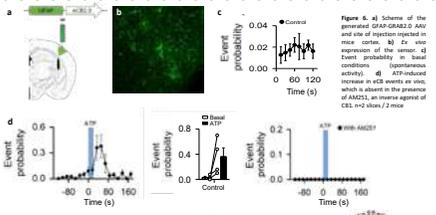


Figure 6. a) Schematic of the generated Spn-GRABeCB2.0 and site of injection. b) Ex vivo expression of the sensor. c) Quantification of the frequency of spontaneous events. d) Quantification of the frequency of ATP-induced events in ex vivo astrocytes, which is absent in the presence of AM251, an inverse agonist of CB1. n=2 slices/2 mice.

CONCLUSIONS

- We effectively produced valuable evidences on astrocytic eCB dynamics in terms of eCB identity, mechanism and outcome. In particular:
- We confirmed that GRABeCB2.0 detects specifically eCB signals in astrocytes, since the activity is absent in the mutated form (GRABeCB2.0mut).
 - Cultured astrocytes show spontaneous eCB activity without any kind of external stimuli.
 - Both astroglial spontaneous activity and ATP-responses are mediated by 2-AG.
 - Astroglial eCB production depends on cytosolic calcium increases.
 - Astrocytes are also able to release eCBs in cultures.
 - Ex vivo cortical astrocytes show spontaneous activity and ATP-induced responses.
 - Ex vivo cortical astrocyte calcium stimulation induces eCBs signals in neurons.
 - Dagle-expressing astrocytes show an enriched expression of genes related to lipid metabolic processes and linoleic acid metabolism.



INTRODUCTION

The Medial Habenula (MhB) is a sub-ventricular structure placed both anatomically and functionally between brain regions that code aversive stimuli, therefore participating in the modulation of emotions. Moreover, the MhB hosts a variety of specialized cells (e.g., larvocyte-like cells, mast cells, and different neuronal and astrocytic phenotypes). In the brain, the cannabinoid receptor 1 (CB1R) participates in the control of neurotransmitter release and has a direct impact on animal behavior. Interestingly, the CB1R is widely expressed in the MhB. We previously showed that global deletion of the CB1R from the MhB results in an impairment of aversive responses. Thus, in this project, we aim to functionally dissect the role of the different MhB cell-types under CB1 control, and to determine how stress-related signals (e.g., immunological, metabolic) acting in the MhB regulate emotional pathophysiology in males and females. We propose that the MhB cellular milieu is a perfect candidate to study the influence of peripheral signals on the pathophysiology of emotional responses.

MATERIALS and METHODS

The CB1R-flux mice (males & females, 7-8 w) were injected with an adeno-associated virus with CRE recombinase, attached to a Green Fluorescent Protein (GFP) tag for its visualization, bilaterally into the MhB. Viral vectors were used to target specifically a certain cell type: GFAP for astrocytes. We tested the innate emotional response (IER) five weeks after the surgery through 3 behavioral paradigms: the open field (OF), the elevated plus maze (EPM) and the light and dark box (LDB). Another group of female mice was injected with LPS (E. Coli lipopolysaccharide) with a single ip injection of 0.41 or 0.83 mg/kg or saline solution to provoke an immune response 24h before the IER. After all experiments, mice were anesthetized and perfused with 4% paraformaldehyde for tissue processing. Using an immunohistochemistry technique, virus administration (GFP/mCherry) site expression were evaluated. A separate group of mice was processed for patch clamp.

VIRAL VECTORS

- *Astrocytes: AAV-9/2hGFAP-mCherry_Cre
- *CTL: AAV-9/2hSyn1-chi-mCherry

LPS injection

Surgery → 5 weeks → IER → 24h → LPS → Protection → f-IHC

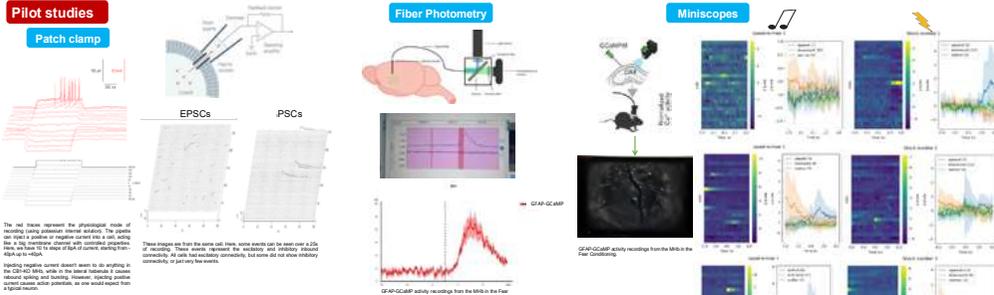
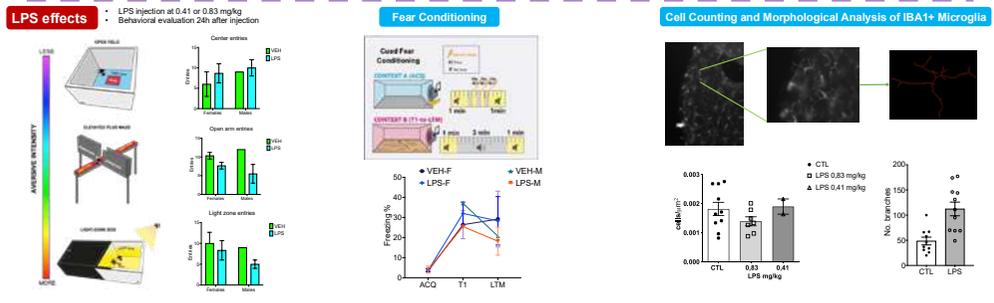
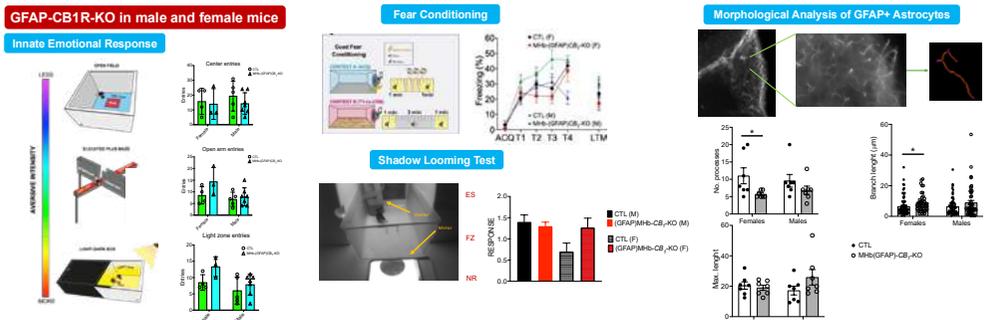
*Thanks to Giovanni Marsicano and Manuel Gumbán.

CannaMetHD LTC incubator

The role of habenular circuits in the pathophysiology of emotions

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 Jon Egaña-Huguet¹
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 Pedro Grandes¹
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SUPPORT

CONCLUSIONS

- Preliminary experiments show that the CB1R expression in astrocytes in the MhB regulate innate and learned emotional behavior in a sex dependent manner.

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INTRODUCTION

Addressing the challenge of antibiotic resistance depends on a deep understanding of the mechanisms driving the emergence and spread of resistance mechanisms, which must be observed in their native environments for effective intervention strategies. Recent breakthroughs in structural biology now enable the direct analysis of microbial communities at different levels of biological organization ranging from the composition and interactions in microbial communities to specific macromolecular complexes they depend on to adapt and survive under stressful conditions. This understanding can provide a base for targeted approaches to combat resistance at its source.

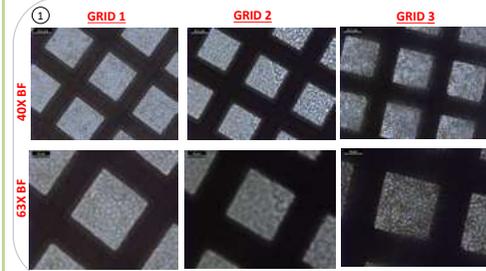
Aim:

To refine *in-situ* structural biology (cryoET) techniques to investigate antimicrobial resistance along the Buetron River gradient, establishing methods for future antibiotic exposure studies.

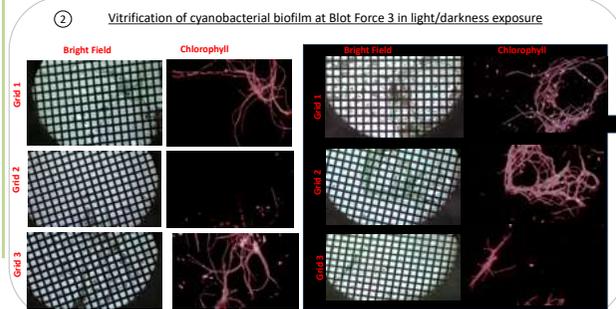
Objectives:

1. Optimize laboratory grown cyanobacteria concentration during vitrification to achieve a uniform monolayer
2. To show that environmental bacteria can be successfully vitrified, allowing lamella to be generated and studied using cryoFIB and cryoET

RESULTS



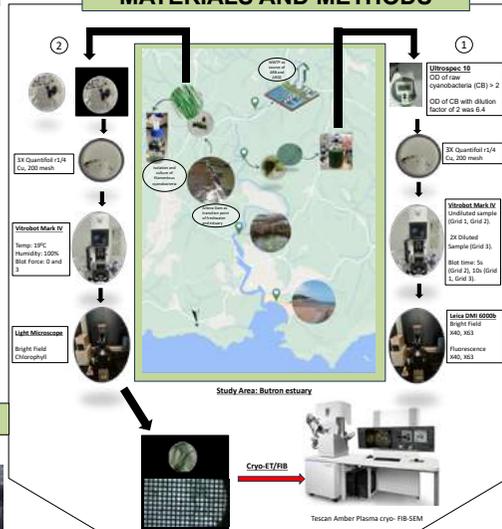
Observation
Grid 3 (Diluted sample) with blot time 10s formed a monolayer.



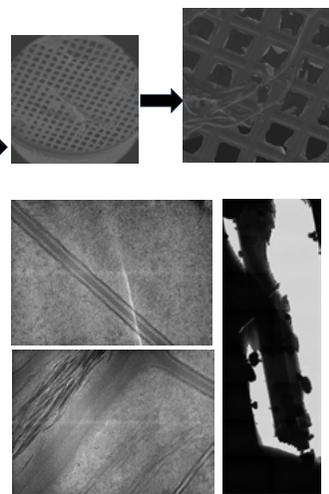
CONCLUSION

- Lab grown cyanobacteria can form a monolayer on cryo-grids for subsequent cryo-ET/FIB processing.
- Cyanobacteria can be isolated as a bacterial mat from the environment and disrupted to allow cyanobacterial filaments to be retained on a cryoEM grid after vitrification.
- Vitrified grids are suitable for cryoFIB milling - we were able to generate thin (approx 200 nm) and stable lamella on the cyanobacteria filaments.
- Our initial studies indicate it is possible to generate vitreous lamella suitable for *in-situ* structural biology from environmental samples

MATERIALS AND METHODS



Lamella generation using cryo-FIB/ET



Acknowledgment: We thank the M3Lab Facility and TRAC expedition of the European Molecular Biology Laboratory for their support. This research is supported by the MINECO grants MCN/AEI/10.13030/501100011033/PI2020-114699B-I001/PI2018-102201/PI2019-102201B-I001, the Basque Government (17/17/22), and the One Health Observational Approach (OH2) in Phenome (PI2020-114699B-I001) funded by MINECO and the European Union. PP and RZ are research fellows under the Ikerbasque program PIPFPA/UPV/EHU.

EAR LTC incubator

Advancing *In-situ* Structural Biology Methods to Investigate Antibiotic Action in Cyanobacteria Mats From Natural Environments

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EXPLORING ENVIRONMENTAL RESERVOIRS OF ANTIBIOTIC-RESISTANT BACTERIA: A CASE STUDY IN THE BUTRON RIVER OF PLENTZIA

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- (5) Structural Biology of Cellular Machines Laboratory, IIS Biobizkaia, Cruces University Hospital, Bilbao, Spain; (6) IKERBASQUE, Basque Foundation for Science, Bilbao, Spain.

INTRODUCTION

The widespread of antibiotics in nearly all environments combined with microorganisms' ability to adapt and survive stress, has led to the emergence of antimicrobial resistance (AMR) [1], which is considered a silent pandemic projected to cause about 10 million deaths annually by 2050 [2]. In relation to clinical, veterinary and food-producing animal settings, AMR monitoring in the environment lack integration and standardization [3]. The identification of environmental AMR reservoirs, where resistant microorganisms can persist, proliferate, and potentially spread to humans and animals, is crucial for establishing routine monitoring and targeting intervention strategies to prevent the spread of resistance in a One Health context [4].

AIM:

Establish a suitable monitoring strategy within the One Health approach to identify ARB's potential sources and environmental reservoirs, understanding their dynamics and dispersal in the Butron River. This under the context of wastewater effluents input in the river, has caused beach closures due to high levels of fecal bacteria indicators [5], while the bay is frequented year-round by locals for aquatic sports and attracts around 20,000 tourists during the summer [6].

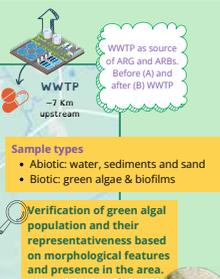
OBJECTIVES:

1. Identification of potential environmental AMR reservoirs within the aquatic ecosystem of the Butron River.
2. Develop a monitoring strategy to identify culturable ARB and evaluate the environmental levels of AMR.
3. Assess the potential of biotic (green algae) and abiotic (water, sediments, and sand) components as hosts of culturable ARB.

METHODS

1. SAMPLING STRATEGY DEVELOPMENT

- Relevant sampling locations
- Antibiotics sources
 - Environmental conditions changes
 - High nutrients load
 - Human exposure



Sample types

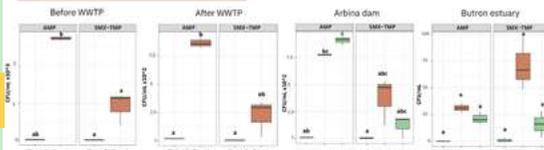
- Abiotic: water, sediments and sand
- Biotic: green algae & biofilms

Verification of green algal population and their representativeness based on morphological features and presence in the area.

RESULTS

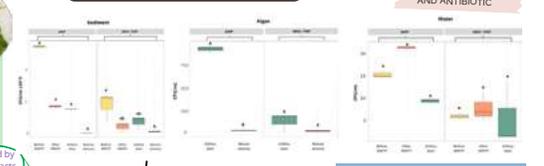
Comparison between environmental reservoirs.

PER ANTIBIOTIC AND SAMPLING LOCATION.



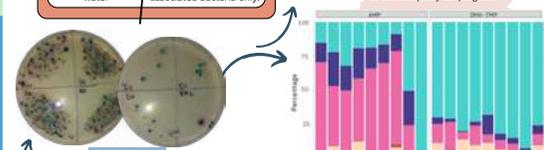
Comparison among sampling sites

FOR EACH RESERVOIR AND ANTIBIOTIC



ARB composition by color colony

Relative concentration (CFU/mL) of each sample by sampling site.



CONCLUSIONS

These findings offer a first assessment of culturable ARBs relevant to human health and suggest the method for estuary samples may need refinement for better microbiome recovery.

Future work

- Improve microbiome extraction for a minimum of 50 µl of 10⁷ CFU/mL *AST
- Narrow isolation of human colonizer/pathogenic bacteria using selective media.
- Monitor ARB seasonal dynamics to assess environmental impacts.
- Metagenomics for ARGs and antibiotic synthesis pathways identification

This work is funded by the MINECO grants MCIN/AEI/10.13039/501100011033 (PID2020-116495RB-I00); PERISST (PID2021-122705GB-I00); Basque Government (IT1578-22) and the One Health Observatory Lighthouse (HOBE) in Plentzia Bay (TED2021-132109B-C21) by MINECO and the European Union. RZ and RP are research fellows under the co-tutelle program PIFPAU-UPV/EHU.

2. ESTABLISHMENT OF BACTERIAL EXTRACTION PROTOCOL

Obtain a representative environmental microbial community with minimal manipulation



3. ANTIMICROBIAL SUSCEPTIBILITY TEST (AST)

Antibiotic	Class	Mode of Action	AST concentration (µg/mL)
Ampicillin (AMP)	Penicillin	Cell wall synthesis inhibitor	8
Doxycycline (DOX)	Tetracyclines	Protein synthesis inhibitor	16
Sulfamethoxazole (SMX)	Sulfonamides	Dihydropteroate synthase inhibitor	76
Trimethoprim (TMP)	Diaminopyrimidines	Dihydrofolate reductase inhibitor	4

CONTRIBUTION OF WATER TO ANTIMICROBIAL RESISTANCE PUBLICATIONS OFFICE OF THE EU

EUCAST CLINICAL BREAKPOINTS

CHROMAGAR ORIENTATION MEDIUM ISOLATION AND DIFFERENTIATION OF PATHOGENS.

Only AST results with over 15 CFU were considered to avoid misleading AMR data. Plentzia beach and DOX excluded.

EAR LTC incubator

Exploring environmental reservoirs of antibiotic-resistant bacteria: a case study in the Butron River, Plentzia

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Ana Luisa Mejía¹
Ransford Parry¹
Itziar Alkorta²
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ICE COUPLING PROTEIN

Ruiz-Cruz, Sofía & Alkorta, Itziar.

Faculty of Science and Technology; Biochemistry and Molecular Biology Department; University of the Basque Country

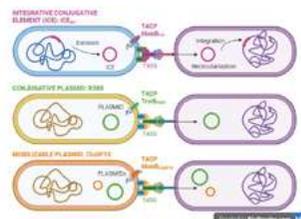


ABSTRACT

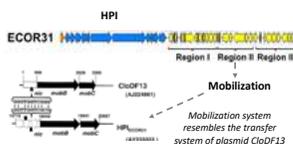
Antibiotic Resistance (AR) is one of the greatest threats to human health. The global overuse of antibiotics has resulted in the proliferation and dissemination of antibiotic resistant bacteria harboring a multitude of AR genes that can be mobilized by different Horizontal Gene Transfer (HGT) processes. Three types of self-transmissible mobile genetic elements participate in HGT: bacteriophages, conjugative plasmids, and Integrative Conjugative Elements (ICEs). Conjugative plasmids and ICEs rely on conjugation for its dissemination, a process that requires direct cell-to-cell contact to enable unidirectional translocation of DNA across the membranes of two mating cells. ICEs are typically found integrated in the host chromosome and encode the machinery for their conjugation, which consists of: (i) proteins that form the relaxosome; (ii) proteins that form the Type IV Secretion System (T4SS); and (iii) the Type IV Coupling Protein (T4CP) that links the relaxosome with the T4SS. To date, ICE coupling proteins have received little attention despite their indispensability in the transfer mechanism. **We are focused on the molecular and functional characterization of the coupling protein an ICE present in multiple *Enterobacteriaceae*, with the ultimate goal of finding ICE conjugation inhibitors to block or, at least, minimize the AR spread.**

BACKGROUND

Conjugative plasmids and ICEs constitute the main drivers of AR spread. Although ICEs outnumber conjugative plasmids, yet they still have been largely overlooked as vectors of AR¹. Both elements encode their conjugation machinery, but conjugative plasmids replicate autonomously whereas ICEs integrate into and replicate along with the chromosome². An integrated ICE remains quiescent, but under certain conditions, the ICE excises from the chromosome as a circular plasmid-like form, which is transferred by the conjugative transfer machinery. Then, is recircularized and integrated in the chromosome³. Additionally, in some cases, ICEs and conjugative plasmids transfer non-conjugative elements such as mobilizable plasmids or integrative mobile genetic elements to a new host.



T4CPs are essential for conjugation, but only a few T4CPs (all plasmid-encoded) have been exhaustively studied, such as the archetype of the T4CP family, TrwB_{R388P}, encoded by the conjugative plasmid R388 or MobB_{CloDF13}, which is one of the few T4CPs encoded by a mobilizable plasmid, CloDF13⁴. Mobilizable plasmids, which can encode AR genes as well, use the T4CPs and T4SS of co-resident conjugative elements, because they usually only encode the proteins needed for relaxosome formation. Given their **critical role in conjugation, T4CPs represent a promising drug target to block conjugation and, hence, AR dissemination among bacteria.**



Figures adapted from 5 and 6.

A homologue of *mobB*_{CloDF13P}, herein named *mobB*_{ICE}, was identified within ICEE1 from *Escherichia coli* ECOR31⁵, containing a wide distributed High-Pathogenicity Island (HPI) among *Enterobacteriaceae*. encodes a functional T4SS similar to the one described for the conjugative plasmid R6K, but its DNA-mobilization region is related to plasmid CloDF13. **The expected product of *mobB*_{ICE} (629 amino acids) shows 31% of identity and 42% similarity with MobB_{CloDF13}.** It was shown that *mobB*_{ICE} is involved in the transfer of a plasmid carrying the origin of transference (*oriT*) present on ICEE1⁶.

*MobB*_{ICE} is an ideal candidate to in-depth characterize an essential component of an ICE conjugative machinery, a T4CP, to find ICE conjugation inhibitors, with the aim of blocking/ minimizing AR spread.

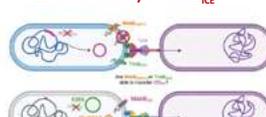
OBJECTIVES

1. *In vitro* characterization of MobB_{ICE}



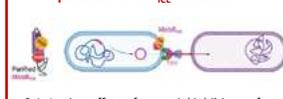
- 1.1. Protein purification.
- 1.2. Determination of oligomerization state.
- 1.3. Effect of the ionic strength, detergent concentration, and pH on MobB_{ICE} stability.
- 1.4. Secondary structure and thermal stability.
- 1.5. DNA-binding properties.
- 1.6. ATPase activity determination.

2. *In vivo* activity of MobB_{ICE}



- 2.1. *mobB*_{ICE} deletion on self-transmission.
- 2.2. Effect of MobB_{ICE} in plasmid conjugation.
- 2.3. Mobilization of CloDF13 by MobB_{ICE}.

3. *In vitro* and *in vivo* validation of potential MobB_{ICE} inhibitors



- 3.1. *In vitro* effect of potential inhibitors of MobB_{ICE}.
- 3.2. Effect of inhibitors on the transfer of ICEE1.
- 3.3. Effect of inhibitors on the transfer frequency of plasmids R388 and CloDF13.

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ACKNOWLEDGEMENTS

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EAR LTC incubator

ICE coupling protein

Sofía Ruiz-Cruz¹

Itziar Alkorta¹

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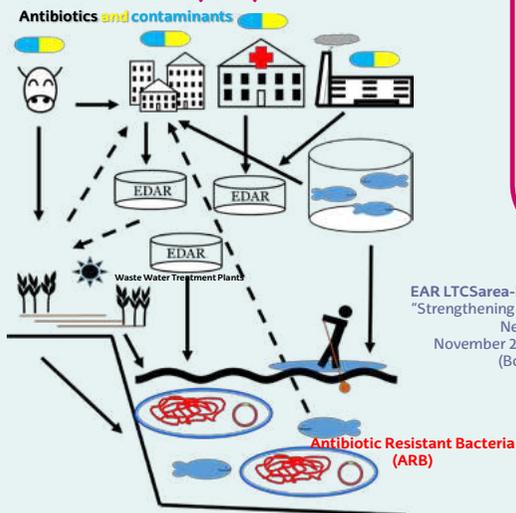
Join the Counter-Resistance team

Antibiotic resistance (AR) represents one of the greatest threats to global health and jeopardizes all the advances in modern medicine. But, although the most dramatic consequences are suffered in the hospital, **the contribution of the environment and the presence of contaminants is one of the main factors aggravating this problem.**

The **solution** therefore lies in an approach to surveillance and action aligned with the principles of the **"One Health"**, in which the problem of antibiotic resistance is addressed at several levels: human, animal, food and environmental.

The main objective of **EAR^{LTC}** is to create a **multidisciplinary network of research groups studying the problem of antibiotic resistance from different perspectives and scientific disciplines, in order to find solutions to this global health problem.**

Environmental Antibiotic Resistance (EAR)



Main objectives

- Collaborative science
- Education
- Outreach

EAR LTCsarea-ENLIGHT CONGRESS
"Strengthening Antibiotic Resistance Networks"
November 2023, HAUT CARRE (Bordeaux)



New Partners are welcome!!



Funding:
Incubadora LTC EAR:
EUSKAMPUS 2023 (EUSK23/01)

EUSKAMPUS 2022 (EUSK22/23)



COORDINATORS
Drs Alkorta and Budzinsky

EAR LTC incubator

Join the Counter-Resistance team

Itziar Alkorta¹

¹ University of the Basque Country and Helene Budzinsky, University of Bordeaux.

From bones to landscapes: Integrated approaches with virtual tools for the study of Neanderthal origins

The LTC incubator HUMEVOL team

One of the main fields of interaction and ground for networking of researchers of the HumEvol LTC incubator is the mutual organisation of fieldwork operations, the development of new research approaches on the field of archaeology, and the understanding of human past.

Two archaeological sites were investigated in 2024 benefiting from a transfronter and transdisciplinary approach in the frame of the LTC Incubator framework: La-Chaise-de-Vouthon (Charente, France), Sima I de el Polvorin (Bizkaia, Basque Country). These two sites are key for understanding the evolution of Neanderthals, and virtual tools are at the centre of the research projects, providing an innovative and revolutionary approach for the development of integrated research in human evolution, the transfer of knowledge and the possibilities of networking.

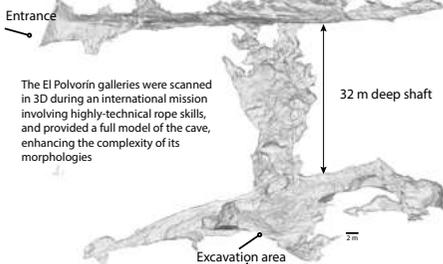


Sima I de el Polvorin

Since 2021 a new archaeological intervention started in the Sima I gallery of El Polvorin cave after the discovery of Neanderthal remains amongst osteological material recovered by speleologists during their explorations in the end of the XX century.

In order to understand how these deposits and remains came inside the cave and into the lower gallery, we started an integrated study combining high-resolution fieldwork at the scale of the excavation, as well as study of the cave system by itself through the use of virtual tools as 3D models.

3D acquisition



The El Polvorin galleries were scanned in 3D during an international mission involving highly-technical rope skills, and provided a full model of the cave, enhancing the complexity of its morphologies

Surveying the excavation



Excavations are performed underground, and will be reported on the 3D topography of the cave, in order to better understand the modalities of sedimentation of the fossil remains

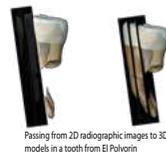
Neanderthal remains



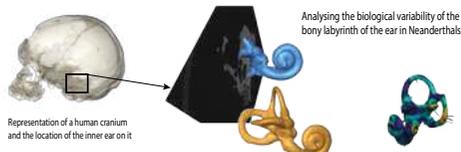
So far, the minimum number of individuals represented in this site is of 1 adult individual: a Neanderthal with archaic features. The general size of the bones is small, so the working hypothesis is that belonged to a female individual.

Looking inside the fossils in 3D

The virtual techniques, and concretely the microCT scanning allows us to see, through a process of segmentation, inside the bones for inner structures and tissue proportions which are of high biological significance and help understanding human evolutionary patterns.



Passing from 2D radiographic images to 3D models in a tooth from El Polvorin



Representation of a human cranium and the location of the inner ear on it

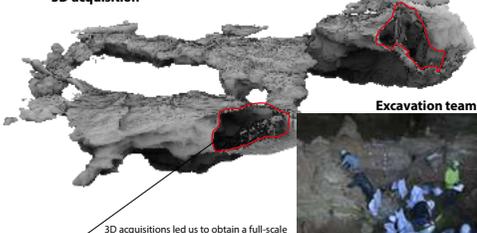
Analysing the biological variability of the bony labyrinth of the ear in Neanderthals

La Chaise-de-Vouthon

La Chaise-de-Vouthon is a classical archaeological site for the knowledge of Neanderthals in Southern France, and the first excavations started around 1870 in the site. This previous scientific history of the site comprises the fact that today some of the original morphologies have disappeared, and that the contextualisation of the remains requires an innovative integrated approach combining multi-scale virtual tools and datings.

The application of this approach will allow us to refine the chronology of the human deposits, reconstruct the formerly excavated deposits and thus understand micro-evolutionary specificities of Neanderthals.

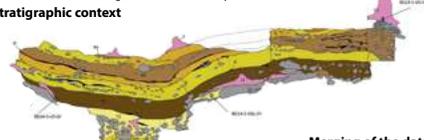
3D acquisition



Excavation team

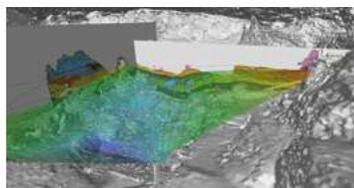
3D acquisitions led us to obtain a full-scale model of the cave morphologies, including the former excavation space

Stratigraphic context



Merging of the data

The combination of the topographical, stratigraphical and archaeological data led us to estimate and reconstruct the ancient morphologies of the cave deposits, during the ancient excavations. We can now reach new understanding of the chronology of the Neanderthal remains



HumEVOL LTC incubator

From bones to landscapes: Integrated approaches with virtual tools for the study of Neanderthal origins

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Both these research projects that benefit of the HUMEVOL LTC incubator frame benefit from the participation of permanent researchers, young researchers, PhD candidates and Master students from both The University of the Basque Country and University of Bordeaux.

Together with the novel and revolutionary approach these projects are core to the networking of the HUMEVOL project, and provide the possibility of multidimensional transfer of knowledge and formation of young researchers. The successful results obtained make us think that this might become the ideal frame to influence the learning of archaeology and become an international and influent school for Human Evolution studies.

Complementary funding:



S5. TECHNOLOGY AND PRODUCTION

From Jerez to the Atlantic: first archaeometric approach to Southwestern Andalusian ceramic trade containers production

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1. Introduction

In the framework of archaeological research on the **Early Modern Age** in the Iberian Peninsula, research is challenge by Castilian ceramic production and understanding its technological changes. In the city of Jerez, pottery production has been documented since the 16th Century including **kitchen ware**, common **domestic unglazed and glazed green and honey-coloured glazed vessels**¹. In the year 2000 the fill of vaults was excavated in the Convent of Santo Domingo. The registered collections are production waste linked to firing processes "loza quemada", displaying 15 forms belonging to the early phase and 25 forms to a late phase². The date assigned to these "jarras" is too late, contradicting data known for Seville, the main production center of these ceramic trade containers.

1. Know the **production centers and technological features**

2. Identify **technical changes over time**

3. The **incorporation of ceramic containers in the first Atlantic routes**

2. Historical Background of Jerez de la Frontera (15th-16th) and the Convent of Santo Domingo

Jerez was part of the territory of the Kingdom of Seville, which in the 15th Century grew and consolidated within the Crown of Castile. Agricultural systems together with cattle raising reached its peak with the fairs of nearby maritime settlements. Those held in Jerez privileged the development of a **winemaking** tradition and the incorporation of **manufacturing artisans** was key to the urban economy.

The foundation of the Dominican Convent, first of several monastic centers after the Christian Conquest under the rule of Alfonso X has had different functions, used as a **warehouse** and as a **defensive building**³. Fill made with **vestibals** in the construction of the Gothic cloister of the Convent (1436 and 1640) has been identified in the vaults lighting system and belongs to two construction phases.

3. Results and preliminar conclusions

Raw materials (rocks and minerals) of the olive jars (*botijas*) are of **sedimentary origin: sandstone, limestone, metamorphic schist**, probably from the Guadalquivir Basin in the **Lower Andalusia** (Seville and its surroundings).

✓ Ceramic forms such as **Dolias and Jars** show raw materials were characteristic of the geological environment belonging from Jerez de la Frontera: **fine red sand, calcareous soils and microfossils** (Foraminifera type).

✓ **Technologically** they are well-manufactured pieces and pastes with a **diversity of colors and shapes**.

✓ **Homogeneous temperature firing**.

✓ The Jerez wine and oil development had an impact on rural life and the **Atlantic diffusion of ceramic containers**.

✓ The **typological identification** of the construction fillings allows establishing the **chronology** of the Convent of Santo Domingo.

✓ This study complements the archaeological and historical knowledge of **ceramic production and ports** in the Atlantic expansion during the Modern Age.

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- 2 Krogan, J. M., *The Spanish olive jar. An introductory study*, *His University Publications in Anthropology* 62, New Haven, 1965.
- 3 Amores Carredano, F. and G. Chaves Jiménez, "Tipología de la cerámica común bajomedieval y moderna sevillana (siglos XV-XVIII). I. la base cuadrada de refresco de bóvedas." *SPN*, 2, 1993, pp.269-325.
- 4 Guerrero Rivero, S. M., 2019. España y construcción en la Arquitectura Religiosa Medieval de Jerez de la Frontera (s. XII-XV). Editorial Universidad de Sevilla, Spain.

5. Acknowledges

- ✓ CERIBAN Grant P202000-EE18R08-B30 funded by MCIN/AEI/10.13039/501100011033 and by "ERDF A way of making Europe" by the "European Union".
- ✓ María Zambrano grant for the attraction of international talent NextGenerationEU (M2AM23/21).

MultiArchSciences LTC Incubator

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Sugar production in Cape Verde: archaeometrical characterization of Trindade sugar pots

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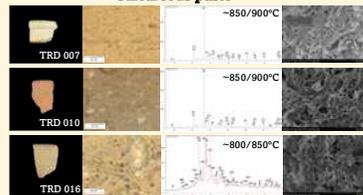
Cape Verde was an uninhabited Atlantic archipelago when it was discovered and occupied by the Portuguese in 1460. During the 15th and 16th centuries, the Portuguese tried to implement the cultivation of sugar cane in the archipelago, although due to Cape Verde's arid tropical climate, it was not as successful as in Madeira, São Tomé and Brazil.



A set of 16 ceramic loaf moulds were analysed by means of chemical (ICP-MS), mineralogical (X-ray diffraction) and microscopic (Scanning Electron Microscopy and Optical Microscopy) methods.



Calcareous paste



Three calcareous fabrics according to EFT and sintering state, including from cream, beige to light orange pastes.

The TRD016 individual, light beige paste, has a very distinctive configuration because an abundance of vitreous material phase as well as primary clay minerals (Illite). Moreover, it shows well developed hematite and calcite peaks.

Low calcareous paste

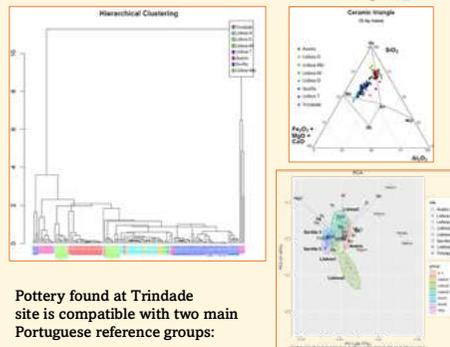


Clearly visible illite peaks along with the presence of K-feldspars and plagioclase (e.g. albite). Calcite is also well developed. Paste color is cream or pale beige.

No calcareous paste



One orange (TRD011) and one red non-calcareous fabric. The first one shows some extent of illite decomposition and the appearance of k-feldspars and hematite. TRD015 shows an intense red paste color, identifying the presence of developed peaks of hematite, quartz and feldspar.



Pottery found at Trindade site is compatible with two main Portuguese reference groups:

- Lisboa: including low calcareous sugar pots (CaO: 0,44% wt) and 3 calcareous possible jug types (CaO: 10,92% wt)
- Aveiro (1 sugar pot) (TRD015)

There are 4 calcareous ceramics that do not match any of the known reference groups (TRD006, TRD013, TRD014, TRD016).

Final Remarks

The results of this research allow us to deepen into this type of ceramic productions and trade in the Atlantic Iberian colonial period.

Trade pots in Cabo Verde sugar industry seems to point out towards a Portuguese origin, mainly from Lisbon region and, at some extent also from Aveiro. However, there is a smaller number of items of unknown origin.

This study piles up information on the manufacture and physico-chemical characteristics of sugar pots found in Cabo Verde.

Acknowledgement

Predoctoral contract FPI PRE2021-097293 and CERIBAM Project (Grant PID2020-113198GB-I00 funded by MCIN/AEI/10.13039/501100011033 and by ERDF A way of making Europe", both funded by MCIN/AEI/ 10.13039/501100011033

MultiArchSciences LTC Incubator

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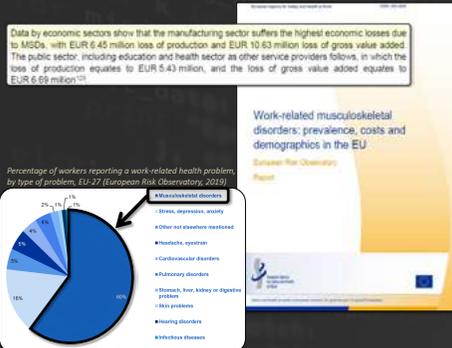
⁶ Archéosciences Bordeaux: Matériaux, Temps, Images et Sociétés - UMR 6034.

Digital twins for occupational health RISK assessment of advanced aerospace manufacturing processes (DI-RISK)

Dr Eirini Konstantinou,
Marie Curie Postdoctoral Researcher, CFAA (UPV/EHU)



Problem Statement



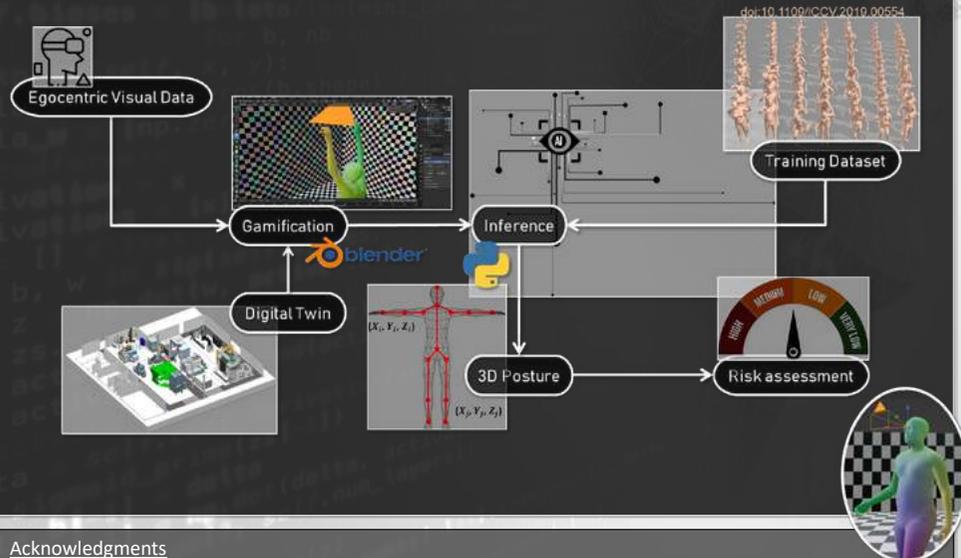
Motivation & Objectives

DI-RISK aims to **assess** those work-related **occupational health risks** affecting aerospace engineers or workers, overcoming the discomfort and lack of accuracy of existing methods.

The specific objectives of this project are:

- a) to **devise** a method and framework that will perform a remote (tag-free) monitoring of multiple engineers under several challenges, such as illumination variations and occlusions, while protecting privacy by design,
- b) to **develop** a method that creates an accurate digital twin (replica) of the human body in order to extract anthropometric and biomechanical data, and
- c) to **convert** anthropometric and biomechanical data into occupational health risk factors in an automated way.

Methodology



Acknowledgments

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Digital twins for occupational health RISK assessment of advanced aerospace manufacturing processes (DI-RISK)

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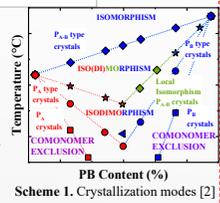
Discovering new crystallization modes in random copolymers

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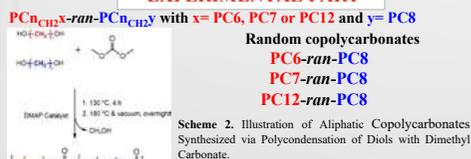
INTRODUCTION

Random copolymers formed by semi-crystalline parent homopolymers can crystallize in three well-known modes (Scheme 1), depending on the comonomer inclusion/exclusion balance: **isomorphism** (total inclusion), **isodimorphism** (partial inclusion), and **comonomer exclusion** (total exclusion). Isodimorphism and isomorphism have garnered significant attention because of their crystallization across all composition ranges. A model Poly(A)-ran-Poly(B) copolymer can illustrate their differences



In isomorphous copolymers, the similarity between PA and PB allows co-crystallization in unique $P_{A,B}$ crystals that differ from the parent components, leading to a linear relationship between the melting temperature (T_m) and comonomer content. For isodimorphic crystallization, due to the comonomer exclusion/inclusion balance, PA-type or PB-type crystals are formed, depending on the composition. The partial exclusion of B co-units causes a T_m depression of PA-type crystals as B content increases, and vice-versa, reaching a minimum T_m , where PA- and PB-type crystals can coexist. This pseudo-eutectic behavior extends to other properties such as enthalpies (ΔH) and crystallinities (X_c). [1,2] This work on a series of linear aliphatic polycarbonates (PC) based on poly(octamethylene carbonate) (PC8) as a fixed comonomer with a second comonomer, a PC, with different chain lengths: PC6, PC7, and PC12 reported apparent pseudo-eutectic behavior of T_m vs. PC8 content, but for specific compositions, their ΔH and thus X_c deviate from the expected behavior. Wide Angle X-ray scattering revealed the presence of a **new third phase (γ phase)** for these compositions, differing from that of the parent components. Further examination of these compositions' properties reveals isomorphous crystallization features. Therefore, our study discovered a **novel crystallization mode for random copolymers that exhibit mixed isodimorphism for some compositions and isomorphism for others.**

EXPERIMENTAL PART



RESULTS AND DISCUSSION

a. Thermal transitions showing pseudo-eutectic trend.

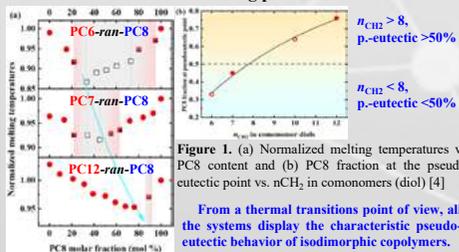


Figure 1. (a) Normalized melting temperatures vs. PC8 content and (b) PC8 fraction at the pseudo-eutectic point vs. nCH_2 in comonomers (diol) [4]
 From a thermal transitions point of view, all the systems display the characteristic pseudo-eutectic behavior of isodimorphic copolymers.

The range of compositions with the γ phase and the pseudo-eutectic point depends on the chain length.

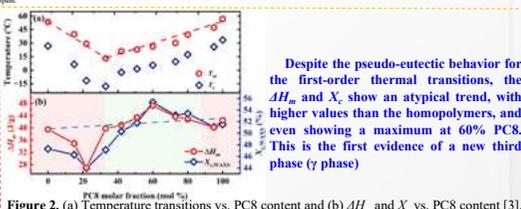


Figure 2. (a) Temperature transitions vs. PC8 content and (b) ΔH_m and X_c vs. PC8 content [3].

b. Structural evidence of a third new phase.

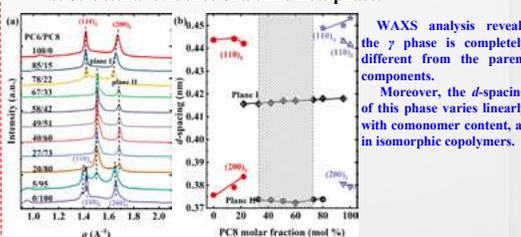


Figure 3. (a) WAXS patterns at -40°C after cooling (at $20^\circ\text{C}/\text{min}$) from the melt. (b) d -spacing vs. PC8 content [3]

c. Isomorphism for specify compositions.

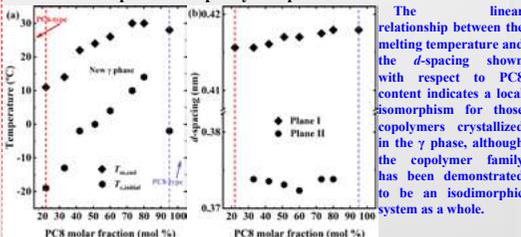


Figure 4. (a) Temperatures and (b) d -spacing vs. PC8 content for compositions with the third new phase. [3]

CONCLUSIONS

- The mixed isodimorphic/isomorphous crystallization mode were found in three different copolycarbonates, and it is characterized by a pseudo-eutectic behavior from a first-order thermal transition point of view but displaying a third new phase for specify compositions from a structural point of view.
- On the one hand, those compositions exhibiting the third new phase exhibit atypically high X_c values that deviate from the expected pseudo-eutectic behavior. Additionally, the range of compositions in which the new phase is present depends on nCH_2 .
- The formation of the new phase can be related to a conformational distortion of methylene segments that facilitates a resurgence of dipole-dipole interactions compared to the compositions where the new phase is absent (according to FTIR results not shown here).

ACKNOWLEDGMENTS

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ADAGIO

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[1] Pérez-Camargo, R. A. *Eur. Polym. J.* 2018, 101, 233 [2] Pérez-Camargo, R. A. *Polymer* 2023, 287, 126412.

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Electrically triggered zwitterionic polymers for Atmospheric Water Harvesting (E-PolyZwit)

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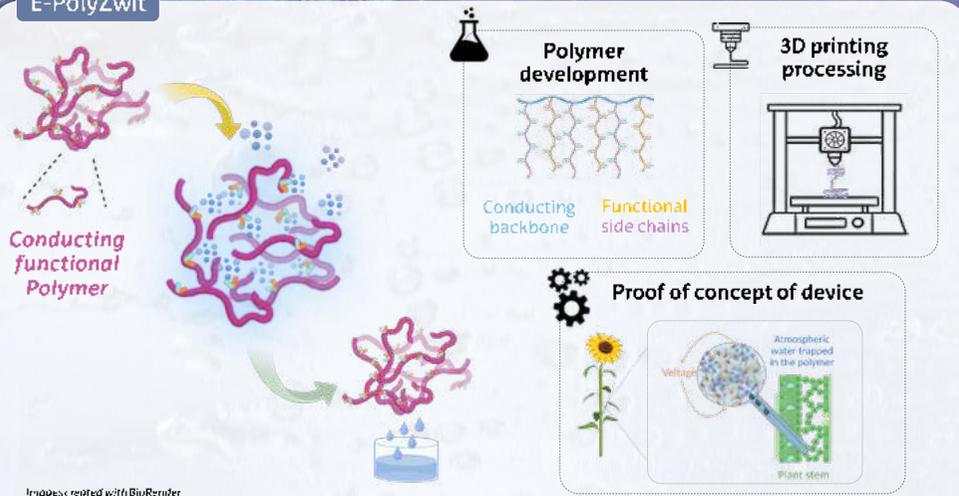
Research need

Water scarcity impacts crop growth and the economy



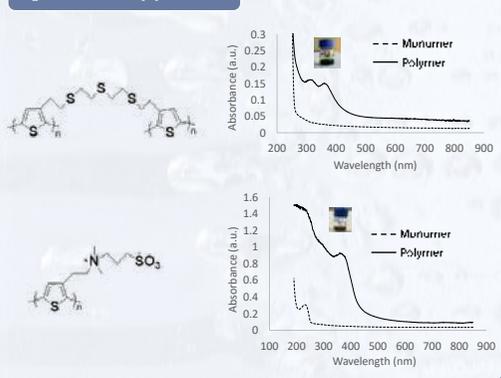
Need for Atmospheric Water Harvesting

E-PolyZwit

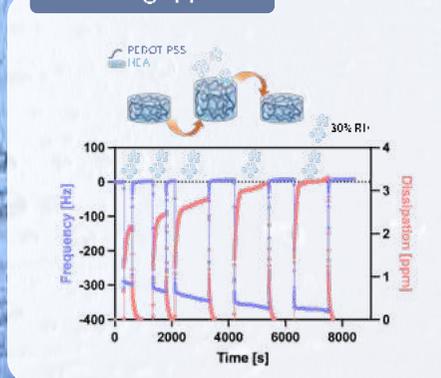


Images created with BioRender

Synthetic approach



Processing approach



ADAGIO

Electrically triggered zwitterionic polymers for water harvesting (E-PolyZwit)

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Investigating Sequence-Structure-Activity Relationship in Foldamer Catalysis

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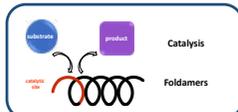


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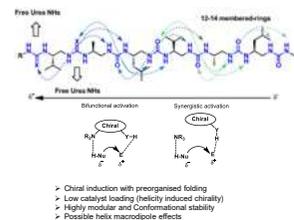
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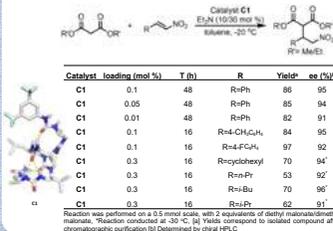


The ability to synthesize artificial sequence-based oligomers that fold with high fidelity (i.e. foldamers) raises new prospects for developing strategies in asymmetric catalysis.[1] In a previous account, we have shown that enantiopure foldamers can be used as H-bond donor organocatalysts in enantioselective C-C bond transformations.[2] These results were recently rationalized by DFT studies which provided a first insight into the origin of enantioselectivity and synergistic activation at the two available catalytic NH sites.[3] However, there is still a lot more to understand about recognition of electrophilic and nucleophilic species by H-bond donor sites to further expand the scope and utility of this catalytic system. In this regard, we have now synthesized new sets of foldamers to study the sequence/structure/activity relationship with several model reactions. The study involves the introduction of different H-bond donor groups at key catalytic sites, modification of lateral side chains and change in the configuration of monomeric units. The effects of these modifications on catalysis will be reported.

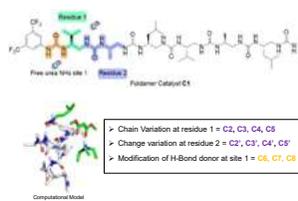
Concept: Catalysis By Helical Oligoourea



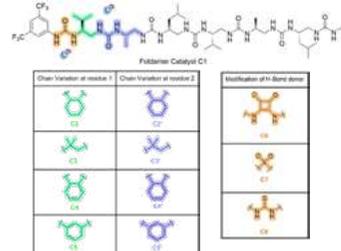
Previous results



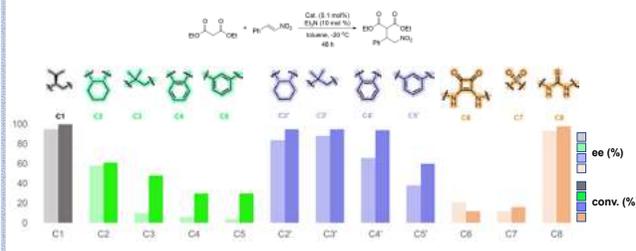
Sequence-Structure-Activity Relationship Study



New Oligoourea foldamers



Catalytic results: Conjugate addition of malonate to Nitro-olefins



Crystal structures of New Catalysts



Foldamer	C1	C2	C3	C4	C5	C2'	C4'	C6	C7	C8
N1-C2'-N1 (torsion)	62.13 ^a	53.1 ^a	-69.8 ^a	-5.5 ^a	-7.8 ^a	65.5 ^a	50.8 ^a	68.8 ^a	63.8 ^a	67.5 ^a
d(N1-N2) Å	5.5	5.4	6.7	6	8	5.8	4.8	6.9	6	6

Addition of enolizable carbonyl compounds to isatin derived ketimines



Entry	Foldamer (mol%)	Branched base	T (°C)	conv. (%) ^a	ee (%) ^b
1	C1 (0.5)	Et ₃ N	-4	85	10
2	C2 (0.5)	Et ₃ N	-4	82	0
3	C4 (0.5)	Et ₃ N	-4	90	62
4	C4 (0.5)	Et ₃ N	-4	90	34
8	C8 (0.5)	Et ₃ N	RT	88	42
6	C8 (0.5)	Ph ₃ EtN	-4	83	77
7	C8 (0.5)	Ph ₃ EtN	-25	87	90
9 ^c	C8 (0.1)	Ph ₃ EtN	-25	90	88

Conclusion & Perspectives

> Design and synthesis of new urea-based foldamers and evaluation of their catalytic activity in a model reaction.
 > Certain residue variations (e.g. C3 and C5) lead to partial helix unwinding at the catalytic site and thus are detrimental for enantiocontrol and catalytic activity.
 > Substitution of the terminal urea by other H-bond donor groups like squaramide (C6) or sulfamide (C7) was not tolerated, despite retention of the helical conformation.
 > The isostructural *cis*-cyclohexyl diamino residue substitution at the first position (C2) results in reduced catalytic activity and enantiocontrol suggesting that some degree of conformational flexibility is needed between the two catalytic sites. Additional computational studies to explain these new results are in progress.
 > We also started to study the Mannich reaction with foldamer C1 and its thiourea version C8. Preliminary results show that C8 actually outperforms C1 in terms of enantiocontrol.

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ADAGIO

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KINETIC MONTE CARLO SIMULATIONS OF MINERAL CARBONATION



Dr. Aleena Alex

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ADAGIO

Kinetic Monte Carlo simulations of mineral carbonation

Aleena Alex¹

¹ University of Basque Country, UPV/EHU.

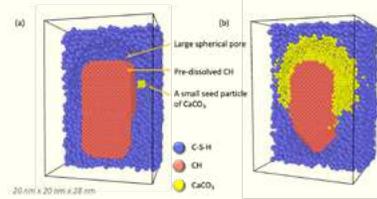
CARBONATION IN CONCRETE

- Carbonation: degradation (lowering of pH, corrosion); Self healing concrete (SHC), carbon capture utilization and storage (CCUS), carbon curing, cement re-carbonation and calcium carbonate engineering.
- Chemical reaction: $Ca^{2+} + CO_3^{2-} \rightarrow CaCO_3$

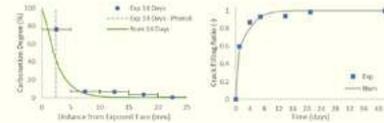


MOLECULAR SCALE CARBONATION

- (a) Initial structure consisting of Calcium silicate hydrate (C-S-H) and Portlandite (CH) and a spherical capillary pore with a seed $CaCO_3$ particle.
- (b) Carbonated structure

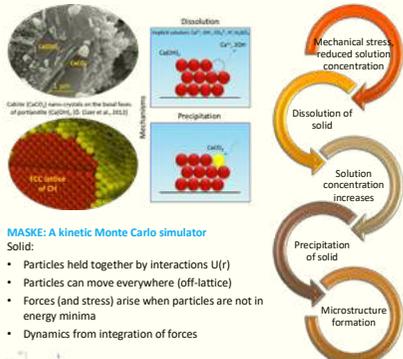


- Effective rates predicted by the molecular scale carbonation simulation were used in macro scale reactive transport and boundary nucleation and growth models and further validated with experimental results.



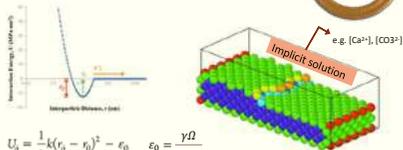
METHODOLOGY

- Discrete element methods: used for mechanical interactions, fracture, strain etc.



MASKE: A kinetic Monte Carlo simulator

- Solid:**
 - Particles held together by interactions $U(r)$
 - Particles can move everywhere (off-lattice)
 - Forces (and stress) arise when particles are not in energy minima
- Dynamics from integration of forces**



$$U_{ij} = \frac{1}{2} k(r_{ij} - r_0)^2 - \epsilon_0 \quad \epsilon_0 = \frac{\gamma \Omega}{n_{disk}}$$

$$\text{Corresponding force: } F_{ij} = -\frac{dU_{ij}}{dr_{ij}} = k(r_{ij} - r_0)$$

Excess enthalpy ΔH_{ex} in the solid \rightarrow solid activity $\neq 1$ (low $U \rightarrow$ more stable \rightarrow low R_{diss})

$$R_{diss} = \frac{k_B T}{h} \frac{c^*}{\gamma} \exp\left(-\frac{\Delta G_{diss}^*}{k_B T}\right) \exp\left[\frac{-\Delta U_{diss}(r) - \gamma \Delta \Omega_{diss}}{k_B T}\right] V_M$$

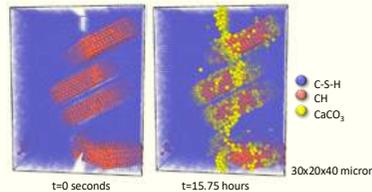
$$R_{prec} = \frac{k_B T}{h} \frac{c^*}{\gamma} \exp\left(-\frac{\Delta G_{diss}^*}{k_B T}\right) \beta V_M$$

Dissolution rate constant k_{diss}

Saturation index: $\beta = \frac{Q_{in,sol}}{K_{eq,diss}}$ Molar volume of particle

MICRO SCALE CARBONATION

- Autogenous healing of the small crack (1–2-micron width) within 15.75 hours (<1 day) of simulated time.
- The effective rates of:
 - CH dissolution: $1.46 \times 10^{-3} \text{ kg/m}^3/\text{s}$ and
 - $CaCO_3$ precipitation: $1.86 \times 10^{-3} \text{ kg/m}^3/\text{s}$



CONCLUSIONS

- Dissolution-precipitation reaction of carbonation in cement paste and microstructure formation simulated using kinetic Monte Carlo theory.
- KMC works at a higher length scale compared to atomistic simulations, each discretized particle representing a molecule. The simulation box sizes can go from nanometer to sub micrometer depending on the computation capacity.
- KMC can sample larger timescales. In KMC time is calculated as inverse of cumulative rate. Thus, depending on the likelihood of dissolution and precipitation, these simulations can sample larger timescales.

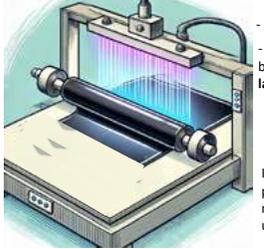
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Leveraging sequential step-growth polymerizations to obtain hierarchically phase separated materials with UV-curing

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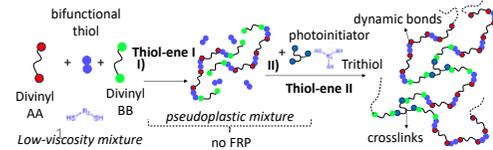
Challenges in lamination of PSA



- Pseudoplastic resin.
- Current free-radical polymerization based PSAs need an N₂ protective layer to avoid O₂ inhibition.

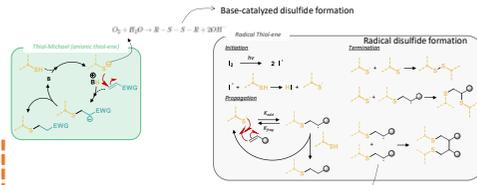
Increased cost, complexity, and provides permanent adhesion, with no capacity of easy removal and re-use

Methods, Strategy



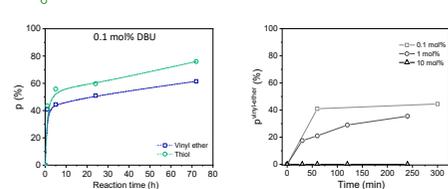
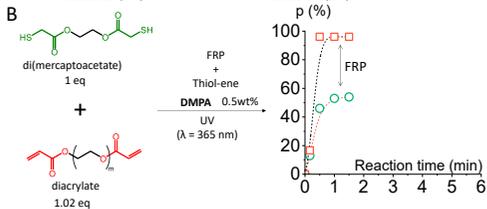
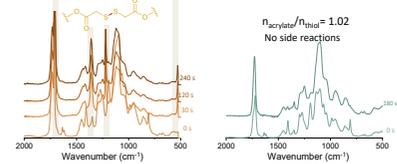
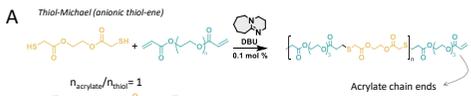
Challenges

- Potential side-reactions

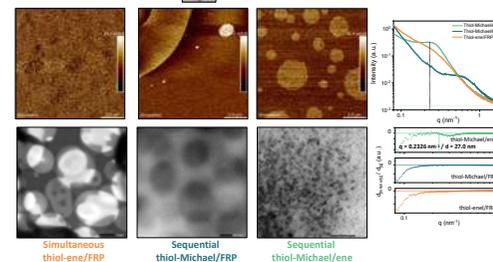
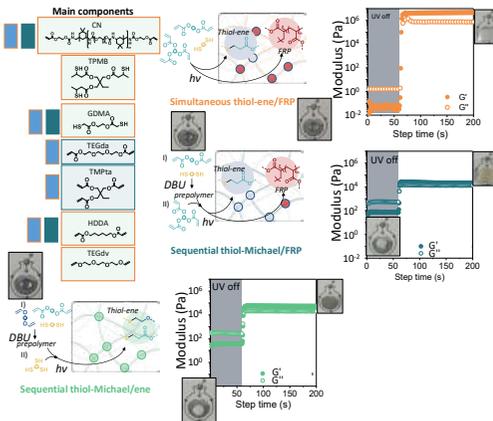


Results

Suppressing side reactions



Three formulations, equal composition, distinct polymerization mechanisms



CONCLUSIONS

- Easy two-step sequential protocol that allow hierarchically phase-separated UV-cured resins with suppression of side reactions and a full step-growth polymerization mechanism.
- Was applied for PSA manufacturing, future applications include 3D-printing bioMedical devices.



This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 101034379

ADAGIO

Leveraging sequential step-growth polymerizations to obtain

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Aritz Iamas¹

Haritz Sardon¹

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Motivations: Hybrid quantum systems comprising a few electrons, such as quantum dots, have garnered increasing attention in cavity quantum electrodynamics due to their potential for realizing ultrastrong light-matter interactions. These systems offer simplified architectures that can significantly enhance and optimize electron-photon coupling. In this study, we initiate by investigating the modifications of the electronic transport within the single and double quantum dot arrays inside the cavity.

Introduction

cavity modification of the material properties

1. Cavity breakdown of the quantum Hall effect [1]
2. Cavity enhancement of the energy transport [2]
3. Cavity control of the topological phase [3]

Aims of the project

1. How cavity modifies the electronic transport of a mesoscopic system
2. Transport properties at strong coupling
3. Interplay between the topology and light

Starting point

Quantum dots provide a better understanding of the electronic transport inside cavity

2. Simple (Single quantum dot without the cavity)

Hamiltonian

$$H = \epsilon_d d^\dagger d + \sum_{k=L,R} \sum_{\sigma} (t_{k,\sigma} c_{k,\sigma}^\dagger d + h.c.)$$

$$J_k = 2eV \text{Re} \left(\sum_{\sigma} t_{k,\sigma} c_{k,\sigma}^\dagger d \right)$$

Current from exact calculation

$$J = \frac{e}{\hbar} \frac{\Gamma_L \Gamma_R}{\Gamma_L + \Gamma_R} \int_{-\infty}^{+\infty} dE \frac{(\Gamma_L + \Gamma_R) / (2\pi)}{(E - \epsilon_d)^2 + (\Gamma_L + \Gamma_R)^2 / 4} (f_L - f_R)$$

Current from rate equation

$$J_k^{(L \rightarrow R)} = \frac{e}{\hbar} (\Gamma_L^L p_{00} - \Gamma_{L \rightarrow 0}^L p_{10}) = \frac{e}{\hbar} \frac{\Gamma_L \Gamma_R}{\Gamma_L + \Gamma_R} (f_L - f_R) \quad k_B T > \Gamma, \epsilon_d$$

The I-V curve obtained using the rate equation approach aligns perfectly with the results from the exact transport calculation based from the Kubo's approach.

3. Complex (double quantum dot without the cavity)

Hamiltonian and tunneling

$$H = \epsilon_1 d_1^\dagger d_1 + \epsilon_2 d_2^\dagger d_2 + t(d_1^\dagger d_2 + d_2^\dagger d_1) + \sum_k (t_{k,L} c_{k,L}^\dagger d_1 + t_{k,R} c_{k,R}^\dagger d_2 + h.c.)$$

$$J = \int dE (f_L - f_R) \Gamma_L G_{11}^r \Gamma_R G_{21}^r$$

Current and transmission

$$J = \frac{e}{2\pi\hbar} \int_{-\infty}^{+\infty} dE T(E) (f_L - f_R) \quad T(E) = \frac{\Gamma_L \Gamma_R}{\Gamma_L \Gamma_R + \hbar^2} \left(\tilde{r}(E - t) + \tilde{r}(E + t) \right) \quad k_B T < \Gamma \ll t$$

$$T(E) = \frac{\tilde{r}^2 \Gamma_L \Gamma_R}{\tilde{r}^2 \Gamma_L \Gamma_R + \hbar^2} \tilde{r}(E) \quad \hbar \ll k_B T < \Gamma$$

4. prospective: adding cavity mode

Hamiltonian for array of dots inside a cavity

$$H = \epsilon_1 d_1^\dagger d_1 + \epsilon_2 d_2^\dagger d_2 + t(d_1^\dagger d_2 + d_2^\dagger d_1) + \sum_k (t_{k,L} c_{k,L}^\dagger d_1 + t_{k,R} c_{k,R}^\dagger d_2 + h.c.) + \omega_c a^\dagger a + \sum_{i=1,2} \lambda_i (a^\dagger d_i + d_i^\dagger a)$$

Complexities

1. Include the cavity modes
2. Include the dissipations

Calculations

1. Exact calculation from Landauer-Buttiker
2. Rate equation for $k_B T > \Gamma, \epsilon_d$
3. I-V curves
4. Utilize it for more complex mesoscopic systems inside cavity

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Acknowledgment

This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 101034379.

ADAGIO

Modifying the electronic properties of the quantum dots with cavity

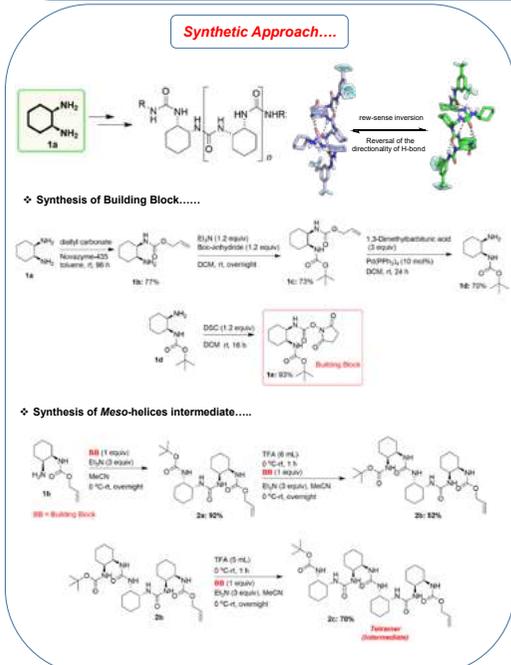
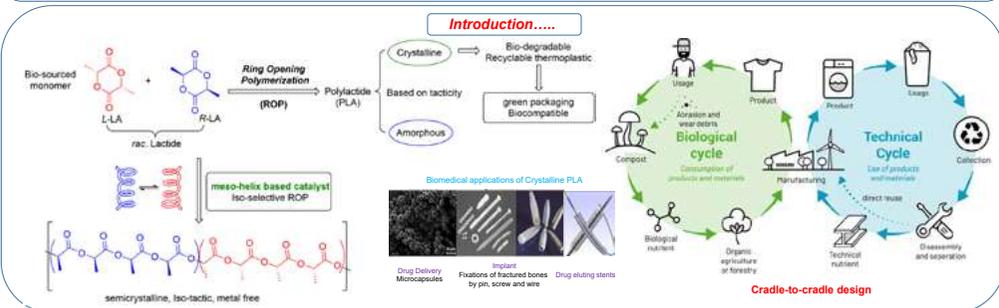
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Synthesis of *meso*- and Pseudo-*meso* Helices via Reversible H-Bond Polarity in Oligourea Foldamers
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Abstract: Poly(lactide) (PLA) is a promising alternative to petroleum-based polymers for biomedical, pharmaceutical, and packaging applications due to its well-known biodegradable, and recyclable thermoplastic nature. PLA's thermomechanical properties, however, are greatly influenced by its crystallinity and hence stereochemistry of its monomer, or tacticity e.g isotactic PLA. While asymmetric organic catalysis using non-metallic catalysts to form C-C or C-X bonds with high stereoselectivity is well established in small molecule synthesis, its application in polymer chemistry remains rare. Even less explored is the adaptation of this concept to stereoselective polymerization, especially using oligourea-based foldamers as catalysts. In this work, we have synthesized dynamic oligourea foldamers, made of *meso* units derived from *cis*-cyclohexane-1,2-diamine as potential catalysts. The resulting *meso*-symmetric sequences are characterized by their ability to equally populate (*P*-) and (*M*-) helices, the two helices being able to interconvert through reversal of hydrogen-bond directionality. Additionally, desymmetrization was achieved by incorporating different terminal functional groups at both ends, leading to a control of helix screw sense. In the future, we aim to study the catalytic potential of these pseudo-*meso* foldamers in the polymerization of *rac*-lactide, with a focus on controlling the stereochemistry of the resulting PLA. In collaboration with colleagues in San Sebastian we also plan to investigate such helical foldamer catalysts in molecular asymmetric catalysis.



Catalyst Synthesis....

Data Analysis....

♦ Crystal structures of (*P*-) and (*M*-) helices
 ♦ Circular dichroism data

3; *M*-helix
 4; *P*-helix

Conclusion:

- To improve existing transformations we have synthesized customizable foldamers.
- We developed dynamic oligourea foldamers as possible catalysts using *meso* units generated from *cis*-cyclohexane-1,2-diamine.
- The resulting *meso*-symmetric sequences are identified by their ability to equally populate (*P*-) and (*M*) helices, with the two helices capable of interconverting via hydrogen-bond directionality reversal.
- We intend to investigate the catalytic potential of pseudo-*meso* foldamers in isoselective ring opening polymerization of *rac*-lactide, with a particular emphasis on regulating the stereochemistry of the resultant PLA.
- We also plan to evaluate these catalysts in asymmetric reactions in collaboration with our colleagues in San Sebastian.

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- [6] Zaky, M. S.; Guichard, G.; Taton, D. *Macromolecules*, **2023**, *56*, 3607.

Acknowledgement:

Collaboration:

- Prof. Daniel Taton, University of Bordeaux
- Prof. Miket Oiarbide, University of the Basque Country

ADAGIO

Synthesis of *meso*- and Pseudo-*meso* Helices via Reversible H-Bond Polarity in Oligourea Foldamers

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1st MEETING OF THE ENLIGHT INTERDISCIPLINARY NETWORK ON THE IMPACT OF GENDER IN HEALTH

Bilbao, Basque Country, Spain
September 16th, 2024

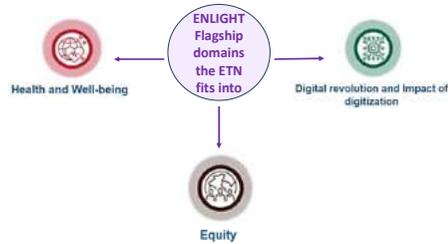
Lucía Gallego, Sandra Sánchez-Urtaza, Laura Alfonso, Elodie Rosanne Santiago & Sofía Villarreal on behalf of the Interdisciplinary Network on the Impact of Gender in Health Faculty of Medicine & Nursing, University of the Basque Country UPV/EHU

The ENLIGHT Interdisciplinary Network on the Impact of Gender in Health <https://impulsenlight.wordpress.com/> is developing the project:

- I** Innovating
- M** Medicine and Health Sciences with Gender
- P** Perspective:
- U** Union
- L** Leadership
- S** Sustainability
- E** Enlightenment

That aims to:

- Raise awareness of the differences that sex & gender factors produce in the expression of health and disease states and healthy ageing
- Promote the integration of sex and gender in health, paying particular attention to (but not restricted to) health education, research, healthcare practice and policy



OBJECTIVES

- To promote an international network to work on the impact of gender inequalities in health and wellbeing.
- To raise awareness of the impact that sex and gender produce in the expression of health and disease states and healthy ageing.
- To address the gaps in biomedical research: (sex and gender inclusion in study designs)
- To raise awareness of the impact of gender-based violence and provide tools for early detection and management.
- To raise awareness of the impact that gender identity and sexual orientation produce in the LGBTBI+ people's health and access to healthcare.
- To integrate these principles into education and help the academic community, especially undergraduate students of Health Sciences, to develop the ability to identify and evaluate health inequalities based on sex and gender in order to design solutions.

GENDER PERSPECTIVE IN THE AREA OF HEALTH SCIENCES INVOLVES

- Making visible and correcting scientifically identified gender biases in relation to the differences between individuals in the response to risk factors, the manifestation and experience of the disease and the differences in health care services.
- Considering these differences has a great impact on new aspects of health:
 - Different response to risk factor exposure
 - Occurrence, incidence and prevalence of diseases
 - Differentiated clinical manifestations
 - Results of treatments, cures and interventions
 - Targeted and improved healthcare
 - Improvement in the clinical management and functioning of health services

NETWORK COMPOSITION

ENLIGHT UNIVERSITIES



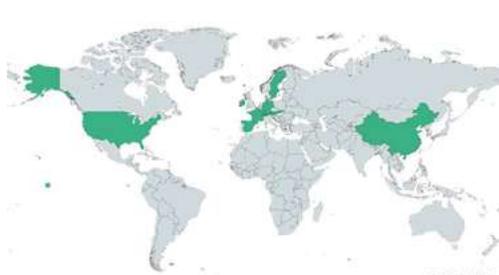
NON-ENLIGHT UNIVERSITIES



OTHER SKATEHOLDERS

- ❖ Students' Union AVIEM, Faculty of Medicine & Nursing, University of the Basque Country UPV/EHU
- ❖ Slovak Pharmacy Students Association, Comenius University Bratislava
- ❖ Student association of the University of Bern
- ❖ Health 4 Future, University of Bern
- ❖ Women's Associations (Basque Country): Asociación Cultural Manuela Equiguren, SARELILAK, ONEKA Asociación de Mujeres Pensionistas, Mairi Emakume Feminista Batza, Eskuz Esku Abesbatza, EMELKA, SIM ROMI Asociación de Mujeres Gitanas, Foro Emakumeak Medikuntzan, Erabide Emakume Elkarte, Etxeko Andre Emakumeen Elkarte, Aldaketa Elkarte Feminista, AMUVES, Hay una esperanza para ti, SIAL
- ❖ Bundesverband Trans e.V.
- ❖ Bundesverband Intergeschlechtliche Menschen e.V.
- ❖ Swiss Gender Health Network
- ❖ Female Empower in Life Science Bern

MEETING STATISTICS



Event countries/states	Event cities
Spain: 8	Spain: 15
Ireland: 2	Ireland: 2
Germany: 2	Germany: 2
France: 2	France: 2
Switzerland: 3	Switzerland: 3
United States: 5	United States: 5
Austria: 1	Austria: 1
China: 1	China: 1
Sweden: 1	Sweden: 1
Slovakia: 1	Slovakia: 1



1st meeting of the ENLIGHT Interdisciplinary Network on the impact of gender in health

Lucía Gallego¹
Sandra Sánchez-Urtaza¹
Laura Alfonso¹
Elodie Rosanne Santiago²
Sofía Villarreal¹

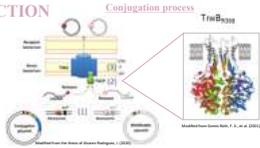
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ABSTRACT

Despite the role of antibiotics in modern medicine, abuse and misuse has led to the emergence of resistant bacteria, being antibiotic resistance (AR) a global health challenge. Type IV coupling proteins (T4CPs) are essential for bacterial conjugation, the main mechanism driving AR spread. Therefore, to control AR spread targeting T4CP emerges as a promising strategy. Here, by proteolysis and electrophoretic mobility shift assays (EMSA) we investigate the interactions between previously identified potential inhibitors and TrwB, T4CP of R388 plasmid. The soluble version of this membrane protein, TrwB₃₃₈ was digested with proteinase K in the presence/absence of the compounds to analyse whether the ligands induce a conformation change, rendering different proteolytic patterns. Some compounds prevented or reduced the protein degradation, indicating that they could be conjugation inhibitors. By EMSA we investigated if the compounds prevent TrwB₃₃₈ from binding DNA. TrwB₃₃₈ was incubated with the plasmid pUC18 in the presence of the compounds, followed by electrophoresis to discern DNA-protein complexes from free DNA. Only one compound induced partial dissociation of the complexes. These experiments are part of work to control the dissemination of AR by inhibiting specifically bacterial conjugation.

INTRODUCTION



Potential inhibitory molecules of TrwB₃₃₈

TrwB₃₃₈ is the T4CP of the conjugative plasmid R388. Following virtual screening (VS), four compounds were selected molecule 1 (M1), molecule 3 (M3), molecule 4 (M4) and molecule 5 (M5).

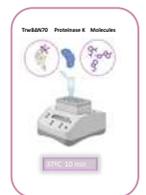


OBJECTIVES

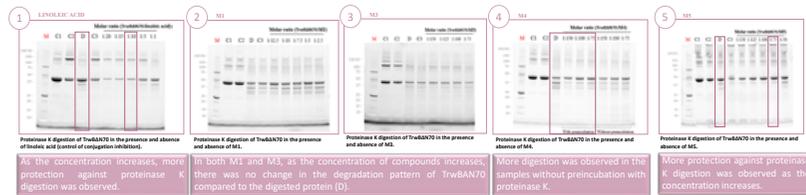
The main objective of this work is to analyse *in vitro* the effect of the potential inhibitors analysed *in vivo*. Molecules M1, M3, M4 and M5 will be validated as ligands of TrwB₃₃₈ through the combination of proteinase K digestion assays and EMSA.

Proteinase K Digestion Assays

MATERIALS and METHODS

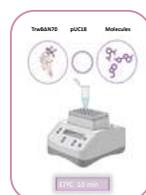


RESULTS

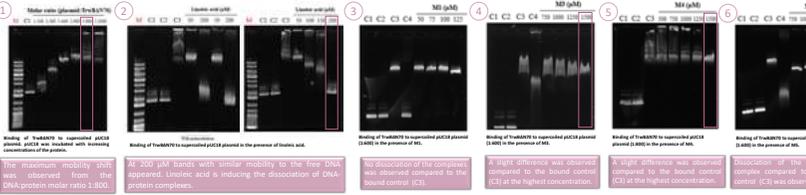


EMSA of TrwB₃₃₈ and pUC18 plasmid

MATERIALS and METHODS



RESULTS



EMSA of TrwB₃₃₈ and a 45-mer oligonucleotide

MATERIALS and METHODS



RESULTS



DISCUSSION and CONCLUSIONS

FUTURE RESEARCH

M1
Internal channel of TrwB₃₃₈
No inhibition was observed

M4
Internal channel of TrwB₃₃₈
Decrease in frequency of 35.9%
Effect in the proteinase K assay

- ### CONCLUSIONS
- The susceptibility of TrwB₃₃₈ to digestion with proteinase K decreases in the presence of linoleic acid, M4 and M5.
 - Linoleic acid and M5 compound can induce the dissociation of the complexes established between TrwB₃₃₈ and pUC18 or the 45-mer oligonucleotide.
 - The *mobICEGTMD* gene has been cloned into the expression vector pET24a (+).

M3
Binding pockets of TrwB₃₃₈ monomer
Decrease in frequency of 40.54%

M5
Inter-monomeric region of TrwB₃₃₈
Decrease in frequency of 32.67%
Induces the dissociation of the complexes

- Induce the G-quadruplex of the oligonucleotide in order to observe M3 effects
- Evaluate the ATPase activity
- The soluble version of TrwB₃₃₈ was used, but these experiments should be also performed with the entire protein
- TrwB₃₃₈ interacts with other molecular players involved in conjugation

Binding Analysis of Potential Inhibitors of Bacterial Conjugation to the Coupling Protein TrwB Using EMSA and Proteinase K Digestion Assays

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CHARACTERIZATION OF THE ANTI-INFLAMMATORY POTENTIAL AND MICROGLIAL BEHAVIOR OF THE CONDITIONED MEDIUM OF DENTAL PULP STEM CELLS

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Characterization of the anti-inflammatory potential and microglial behavior of the conditioned medium of Dental Pulp Stem Cells

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INTRODUCTION

Limitations of stem cells based therapy are: i) large amount of cells needed; ii) risk of host rejection & iii) correct cell integration to avoid uncontrolled cell proliferation.

Recent strategies have focused on the potential of stem cells secretome as it can modulate the physiological state even of distant cells while avoiding this major risks and drawbacks (Mukhamedshina Y. et al. 2019; Nakano M. et al. 2021).

In this work, we analyzed and tested the secretome of Dental Pulp Stem Cells (hDPSCs) as a possible alternative for brain therapy.

RESULTS

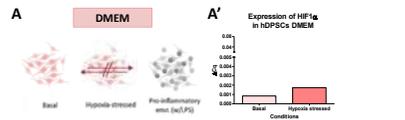


Figure 1. A) Schema of cell culture conditions used in the study: hDPSCs cultured in DMEM in basal conditions either normal or in an hypoxia stressed environment and their (A') HIF1α expression determined by RT-qPCR.

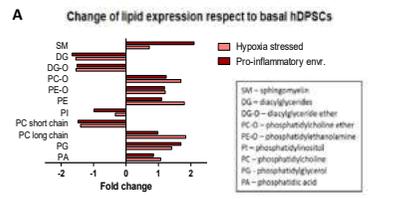


Figure 2. A) Lipidomic analysis showing the relative changes in lipid expression of hDPSC under hypoxia or pro-inflammatory environment respect to basal conditions.

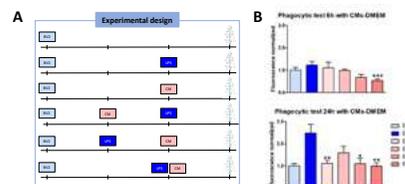


Figure 3. A) Experimental design for the phagocytic test; BV2 microglia cell line was cultivated for 6 or 24 hours with basal media, media supplemented with LPS (20 ng/mL), media supplemented with CMs (800 µg/mL), CMs and after LPS or viceversa, or LPS & CMs at the same time. After the treatments, BV2 were in contact with the beads (2 · 10⁶ microspheres) for 1 hour and 15 minutes, and fluorescence was measured. B) Quantification of the phagocytic capacity of BV2 through phagocytosis of fluorescent beads after 6 & 24 hours, respectively, with each condition (n=4, with triplicates). Statistics respect to BV2 LPS: 6 hours: ***, p<0.0001; Kruskal-Wallis with post-hoc; 24 hours: BV2 LPS & BV2 EVs: **, p=0.0111; BV2 LPS & BV2 LPS-EVs: *, p=0.0108; BV2 LPS & BV2 LPS-EVs: **, p=0.0051; Kruskal-Wallis with post-hoc. Data is represented as mean and SEM.

METHODOLOGY

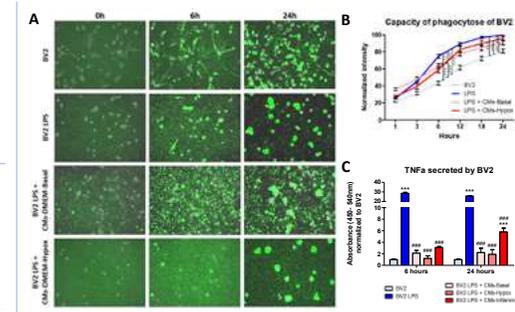
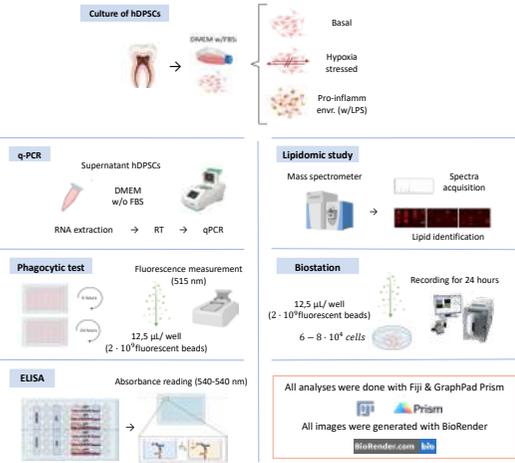


Figure 4. A) Track of the phagocytic capacity of BV2 microglia cell line through phagocytosis of fluorescent beads (2 · 10⁶ microspheres) during 24 hours with basal media and media supplemented with LPS (20 ng/mL), LPS & CMs from the corresponding conditions (800 µg/mL). Image snapshots showing the cells 0, 6 and 24h post-addition of beads, LPS & CMs respectively. B) Quantification of fluorescence intensity mean in cells (n=100-120 for BV2 & BV2 LPS; n=50-70 for BV2 LPS-CMs) and normalized to BV2 with LPS after 24 hours. Statistics 6 hours: ***, p<0.0001; BV2 LPS & BV2 LPS-CMs-Basal **, p=0.0025; BV2 LPS & BV2 LPS-CMs-Hypox. ***, p=0.001; Kruskal-Wallis with post-hoc. Statistics 24 hours: BV2 & BV2 LPS ***, p<0.0001; BV2 & BV2 LPS-CMs-Basal **, p=0.0029; BV2 & BV2 LPS-CMs-Hypox ***, p=0.0009; BV2 LPS & BV2 LPS-CMs-Basal ***, p=0.0002; BV2 LPS & BV2 LPS-CMs-Hypox ns, p=0.2716; Kruskal-Wallis with post-hoc. Significant differences at other time points not shown. Data is represented as mean and SEM. C) Quantification of the levels of TNFα secreted by BV2 microglial cell line under basal conditions, activated with LPS & the corresponding CM, 6 and 24 hours after their addition. Statistics: ***, p=0.0001 respect to BV2; ###, p<0.0001 respect to BV2 LPS; two-way ANOVA test with post-hoc. Data is normalized to BV2 and represented as mean and SEM.

CONCLUSIONS

- **Conditions of culture** (different stresses) change the physiologic state of cells at different levels: membrane lipids, transcribed and packed RNA, etc. In consequence, there are also changes in their secretome that can influence differently other cells in a paracrine manner.
- **Conditioned media from hDPSCs can modulate the state of activation of BV2 microglia.**
 - i) Through a **decrease of their phagocytic capacity** that is maximal at 6 hours and remains during the first 24 hours. However, CMs-DMEM cannot prevent BV2 from activation when they are added before LPS.
 - ii) Through a **significant decrease of TNFα secretion** at 6 & 24 hours for all CMs-DMEM.

FUNDINGS

This work has been funded by the Basque Government (IT1751-22 (G.I. (J.R.P.)), Polimerbio SL (2023.0012 (J.R.P.)), the University of the Basque Country UPV/EHU (COLAB22/07 (J.R.P.)) and the Spanish Ministry of Science, Innovation and Universities (MICIU) (PID2019-104766RB-C21 (J.R.P.) and PID2023-152704OB-I00 (J.R.P. & G.I.)) MCIN/AEI/10.13039/501100011033 by the European Union (NextGenerationEU) "Plan de Recuperación, Transformación y Resiliencia". IMR, SMC obtained a Ph.D. fellowship from University of the Basque Country UPV/EHU (PIFBR21/05 & PIF22/119). BPR, JSM obtained a Ph.D. fellowship from Basque Government (PRE_2023_2_0112 & PRE_2023_2_0038). YP has a Bikaintek PostDoc grant (010-B1/2023).

We want to acknowledge to the SGIKER technicians Ricardo Andrade and Alejandro Díez and Rafael Martínez-Conde's maxillofacial surgery clinic.

Conceptual model to evaluate the practices and uses of the fundamentals of Storytelling in the narratives of corporate websites.



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Field: Social Communication
Director: Sergio Monge

Abstract

The narratives of corporate websites represent windows to the world of brands and organizations that surround the everyday environment. However, depending on how their content is presented, they can go unnoticed, or on the contrary, they can impact, captivate and show a vital commitment to their audiences and society.

Corporate websites are spaces where the energy of the content can transport the public towards paths of emotions and transformations, just as happens with the stories. But, this does not have to do with using literally a story and presenting it chronologically in some section of the website, but rather with studying and analyzing the theoretical foundations of storytelling and then applying them systematically.

Two types of approaches: functional and symbolic.

Results

Methodology

Content analysis

Other elements that were included in the conceptual model:

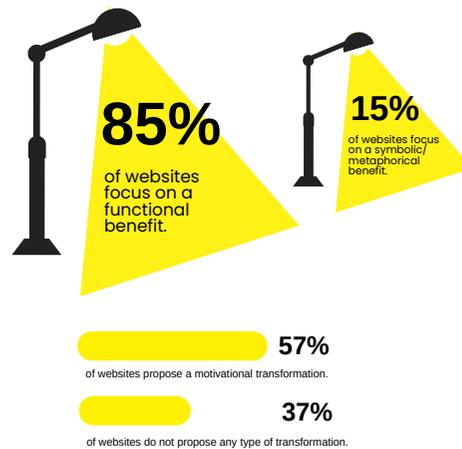
Emotions Plot Goals Archetypes Mental Metaphor

References

Escalas, J. (2004). **Narrative Processing: Building Consumer Connections to Brands.** *Journal of Consumer Psychology*, 14 (1&2), 168-180.
Moin, S. M.A. (2020). **Brand Storytelling in the Digital Age.** London: Palgrave Macmillan.
Spear, S. (2015). **The Role and Significance of Corporate Stories in Corporate Reputation Management.** (Phd Thesis). University of Portsmouth.

Main objective

This research proposes the development of a conceptual model based on the foundations of storytelling to evaluate and analyze its use on the websites of corporations from the ranking of Great Place to Work companies, that is, organizations with a positive global rating regarding their culture and internal communication, but what about their external communication through their websites? Do they generate content that impacts and transcends?



Conclusions

The analysis revealed two approaches: the narrative/symbolic perspective and the functional/rational perspective. The first arises from verifying its connection with key elements from the theory of storytelling. And the second one is associated with more conventional and predictable content.

Thus, storytelling, the ancient art of telling, joins the world of communication to propose improvements and changes in corporate narratives, always starting with the question: how to generate transcendence and meaning?, not only for today, but also for the future.

Conceptual model to evaluate the practices and uses of the fundamentals of Storytelling in the narratives of corporate websites

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How Contrastive Learning overcomes Domain Shift in classification tasks

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Definitions

Domain Shift occurs when the probability distribution of the data used to train a model (source domain - SD) differs from the probability distribution of the testing data (target domain - TD). The goal of modelling is to be accurate in the testing scenario, but a model trained on the source domain is not guaranteed to retain performance in the target domain.

Given a dataset and a notion of similarity between its samples, Contrastive Learning (CL) techniques learn a new representation (the latent space) by bringing related samples closer to each other and maximizing separation to unrelated samples.

Objectives

Define a similarity notion for a binary classification task with two domains, and use CL to reduce the domain shift in the representation.

Defining similarity

- The different classes in the source domain must be apart in the latent space
- Class i from the TD must be close to class i from the SD
- Class i from the TD must be far from class j from the SD

Training & evaluating the representation

- Training**
- Define a representation function: we have used a MLP.
 - Assign a label matrix (Fig. 1) for the data using our similarity notion.
 - Translate raw data to the latent space.
 - Compute a pairwise similarity of all samples in the latent space, and use a sigmoid to express that as a probability.
 - Compute the binary cross-entropy between the pairwise similarity and the label matrix.

	SO	SI	TO	TI
SO	1	0	0	0
SI	0	1	0	0
TO	0	0	1	0
TI	0	0	0	1

Fig. 1: Label matrix

Evaluating

- Baseline: Fit a Logistic Regression (LR) in data from the source domain, in the original feature space. Evaluate it in data from the target domain.
- Evaluation of CL: Use the same instances involved in the baseline, but represent them in the latent space. Fit a LR in the source domain instances and evaluate it in the target domain instances.

Generating synthetic data

Source domain: Classes 0 and 1 come from two overlapping 400-dimensional gaussian distributions (N=3000).

Target domain: The relationship between classes 0 and 1 is maintained, but the mean and scale of the gaussians has changed (N=3000).

The 2-dimensional representation using TSNE shows the domain shift (Fig. 2)

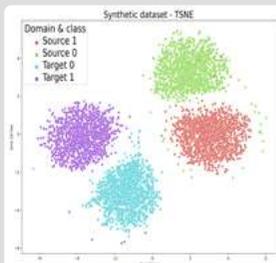


Fig. 2: Visualization of the dataset

Results

Visualization of the latent space

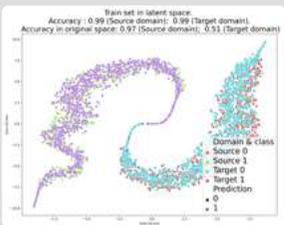


Fig. 3: Latent space representation of instances used for CL training

Fig. 3 shows that the latent space has acquired the desired structure: Source instances from different classes are apart from each other, ensuring a good source classification, and target instances from each class have a similar representation to source instances from their corresponding class, ensuring applicability of the source classifier.

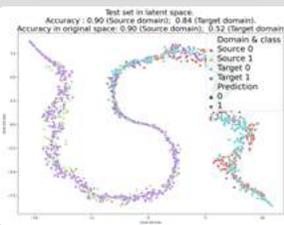


Fig. 4: Latent space representation of instances not used for CL training

Fig. 4 shows that the representation function (MLP) generalizes well to unseen samples: The domain shift is reduced, and the unseen instances form separable clusters in terms of class but not domain in the latent space.

Impact on cross-domain classification

Fig. 5 shows that the new representation significantly increases the accuracy of the source classifier when applied to the target domain.

Blue represents the accuracy across 50 folds of a source classifier applied to the target test set using the original space (the baseline).

Orange represents the accuracy of the source classifier, built and tested on the same data, but using the contrastive latent space.

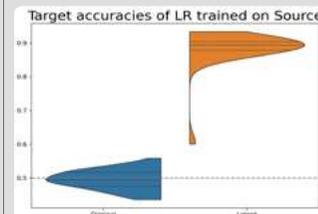


Fig. 5: Classification performance

Conclusions & Future Work

- For the samples used during CL training, the latent space effectively solves the domain shift problem (Fig. 3).
- This generalizes well to unseen samples (Fig. 4).
- Classification results in the target domain improve notably due to the CL representation.
- Moving forward, we will apply this method to real-world scenarios, particularly in the field of Neuroscience.

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How Contrastive Learning overcomes Domain Shift in classification tasks

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Improvement of human dental pulp stem cells neurodifferentiation methods to obtain functional neuron-like cells.

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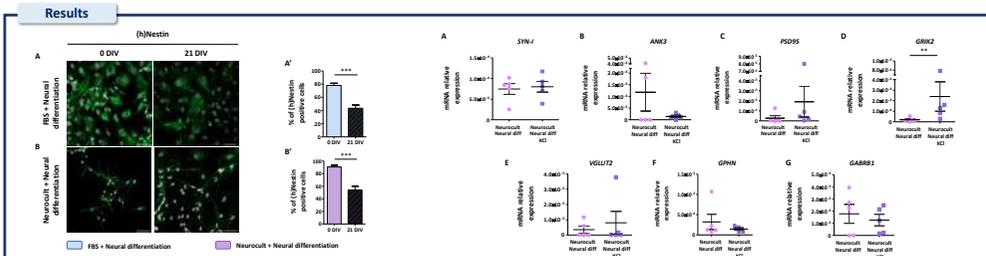
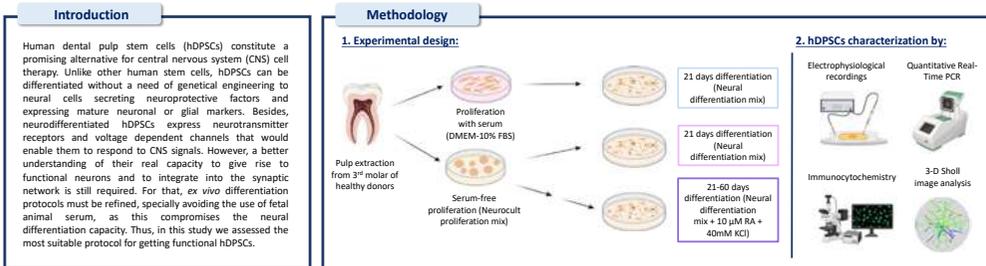


Fig. 1: Human Nestin stem cell marker expression decrease after 21 days of neurodifferentiation. (A) hDPSCs proliferated with serum containing fibroblast like morphologies and detectable decrease of (h)Nestin labelling. (A') Quantification of (h)Nestin positive cells just after switching them from a DMEM-10% FBS media to a neural induction mix and 21 days afterwards. (B) Cells grown in neurocult proliferation mix presenting a ramified morphology and a decrease in (h)Nestin positive labeled cells. (B') Percentage of (h)Nestin positive cells after switching them from a serum-free proliferation mix into a neural induction mix and 21 days afterwards. ***p<0.001. U-Mann Whitney (Two-tailed) test. Scale bar 50 µm.

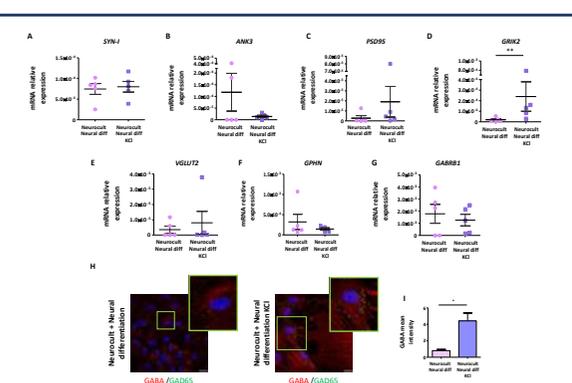


Fig. 4: Pre-synaptic proteins, excitatory glutamatergic and inhibitory GABAergic synapse components expression in hDPSCs after 21 days with or without RA and KCl. (A) axon initial segment protein Ankyrin-G, (C) postsynaptic PSD95, (D) glutamate ionotropic receptor kainate type subunit 2 (GRIK2), (E) vesicular glutamate transporter (vGLUT2), (F) gephyrin (GPHN) and (G) gamma-aminobutyric acid type A receptor (GABRB1) mRNA relative expression in hDPSCs differentiated with the same neural differentiation mix and those in which RA and KCl was added on top of it. (H-H) GABA neurotransmitter and glutamate decarboxylase (GAD-65) (green dots) immuno positive labelling in cells neurodifferentiated without RA and KCl and more intense labelling of those GABAergic markers in those cells that were treated. Scale bar 20 µm. **p<0.01. ***p<0.001. Statistical analyses were conducted by U-Mann Whitney (Two-tailed) test.

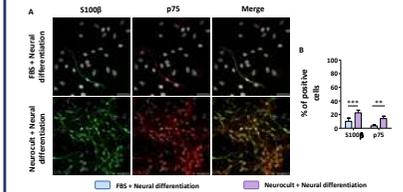


Fig. 2: Neurodifferentiated hDPSCs are able to commit toward Schwann Cell phenotypes (A) Higher rates of immunopositive cells for p75 and S100β could be observed in hDPSCs grown with neurocult comparing to those that were grown with FBS after 21 days of differentiation. Scale bar 50 µm. (B) Graphs showing the percentage difference for S100β and p75NTR markers between hDPSCs that have grown with FBS and those in Serum-free Neurocult media after switching them to the same neural induction mix during 21 days. ***p<0.001. Statistical analyses were conducted by U-Mann Whitney (Two-tailed) test.

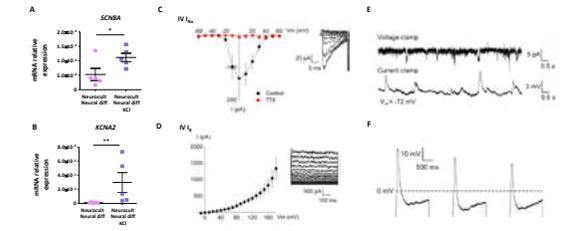


Fig. 5: Voltage dependent Na⁺ (SCN8A) and K⁺ (KCNA2) channel expression and electrophysiological recordings. Graphs showing an increased expression of voltage-gated sodium (SCN8A) (A) and potassium (KCNA2) (B) channels in those cells treated with KCl and RA. *p<0.05, **p<0.01. U-Mann Whitney test or Student's t-test (Two-tailed). Current-voltage relationship of Na⁺ currents from -60 to 60 mV (C) and K⁺ currents from -10 to 180 mV (D) on two months hDPSCs neurodifferentiated with KCl and RA. (E) Spontaneous electrophysiological activity and (F) repetitive action potentials firing patterns in hDPSCs differentiated with KCl and RA.

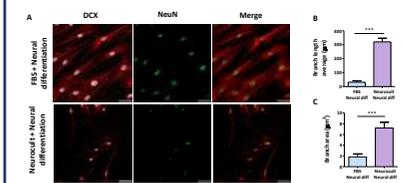
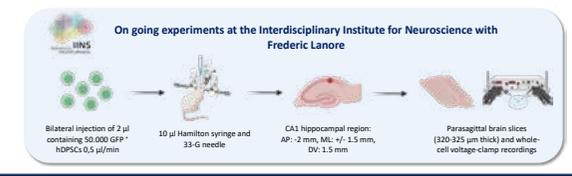


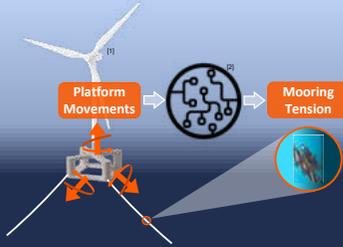
Fig. 3: Neuronal marker expression and cell morphological analysis after 21 days of neurodifferentiation. (A) Immunofluorescence images showing doublecortin (DCX) and neuronal nuclear protein (NeuN) positive labelling in hDPSCs and different morphologies observed between cells grown in a serum containing medium and those that were grown in a serum-free medium. Scale bar 50 µm. (B) 3D Sholl-analysis of 30 cells per condition revealed longer branch lengths measured in µm (B) and a larger overall surface (µm²) (C) occupied by hDPSCs processes in those cells previously grown as floating dendrites. ***p<0.001. Statistical analysis conducted by U-Mann Whitney (Two-tailed) test.



MACHINE LEARNING ALGORITHMS FOR ESTIMATING MOORING LINE TENSIONS OF FLOATING WIND TURBINES

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Virtual sensor based on machine learning shows the potential to replace physical sensor for measuring mooring line tensions but needs to be further improved for sea states underrepresented in the training data.

[1] Source: Tecnalia Research & Innovation
[2] Osarena Leyre (2017). Artificial Intelligence. Available at: <https://doi.org/10.1007/978-84-9017-000-0> checked on 22/09/2024

Machine Learning Algorithms for Estimating Mooring Line Tensions of Floating Wind Turbines

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1. Motivation

- Mooring systems are critical subsystems of Floating Offshore Wind Turbines (FOWTs) that require reliable monitoring.
- Direct measurement of mooring tensions is challenging and expensive due to submerged and harsh offshore conditions.
- Indirect estimation of mooring tensions using machine learning is explored in this study as an alternative approach.
- The floater's motions are used as input, as they are easier to measure and offer greater robustness.



2. Use Case

- Measurements were used from a 1/13.6 scaled wave tank model of HarshLab2, a floating laboratory owned by Tecnalia Research & Innovation.
- 15-min runs of 12 different sea states were conducted in a wave tank.
- The floater's motions in six Degrees of Freedom using an optical tracking system and mooring tensions in the three catenary mooring lines using load cells were measured.



Real scale floating laboratory HarshLab2



Small scale wave tank model of HarshLab2 that provided training data for this study



Chain and load cell of wave tank model's mooring system



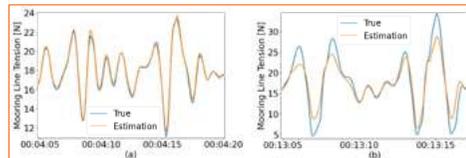
3. Machine Learning Model

- A Support Vector Regressor (SVR) with a nonlinear kernel was used.
- The SVR model was trained on data from nine sea states and tested on three held-out sea states.



4. Results

- The model demonstrated strong performance, achieving an R^2 greater than 92% and an average RMSE below 0.9N for all sea states.
- The model's performance diminished for sea conditions not well represented in the training data, showing limited generalization capability.



Time series segment of estimations (orange lines) and observed values (blue lines) of mooring line tension of test sea states 62 (a), test sea state 69 (b)

Characteristics of sea states in training and test data and corresponding performance metrics of the SVR model

Run N°	H_s [m, real scale]	T_p [s, real scale]	Dataset	R^2 [%]	RMSE [N]
58	1	5.79	train	98.98	0.1405
59	1	7.72	test	92.18	0.2188
60	1.36	9.65	train	98.45	0.0927
61	1.88	6.87	train	99.44	0.1432
62	1.88	9.15	test	98.48	0.1667
63	1.88	11.44	train	98.44	0.1000
71	2.04	14	train	97.97	0.1016
64	3	7.79	train	99.56	0.1923
65	3	10.39	train	99.22	0.1653
66	3	12.98	train	99.21	0.1672
69-70	4.3	12.29	test	94.01	0.7102
67-68	5.6	9.22	train	97.99	0.8792



5. Future Outlook

- For the remainder of our PhD research the identified generalization problem under unknown sea conditions will be addressed.
- Several other use cases are planned to be investigated, such as wave tank models of FOWTs and, if available, real scale devices installed in offshore conditions.



Microscopic insight on cementitious materials using radiation-scattering techniques

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Why concrete?

- Cement is the most used synthetic material, with its global production still increasing.
- Great efforts are needed to develop new and more sustainable mortars.
- Despite centuries of studies, basic questions still remain poorly understood.

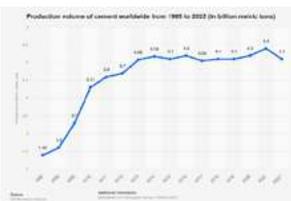


Figure 1: Evolution of global cement production.

Laboratory techniques

- Differential scanning calorimetry (DSC) can be used to study the isotope effect of hydration.

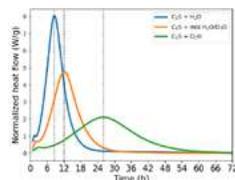


Figure 2: Heat flow evolution of C₃S hydration with different deuteration levels.

- Raman spectroscopy allows the mapping of surfaces of new types of cements.

- This gives information on the main phases composing the material, as shown in figure 3 for a cement that contains portlandite, calcite and a gel phase.

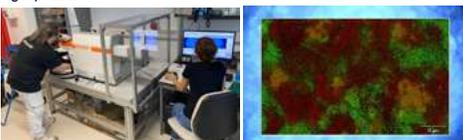


Figure 3: (right) in-Via Reflex Raman by Renishaw (Left) Raman surface mapping of a cement with portlandite (yellow) and calcite (red).

Neutrons (at international facilities)

- Inelastic neutron scattering (INS) can see what Raman can't.

- Figure 4 shows the different lattice and molecular modes of C₃S due to 1 or 2 protons from the hydration.

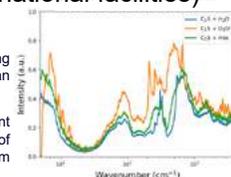


Figure 4: INS spectra of C₃S hydration with different deuteration levels

- Neutron imaging is used to analyze the structure of a material.

- Figure 5 shows the structure of cement foam (CF).

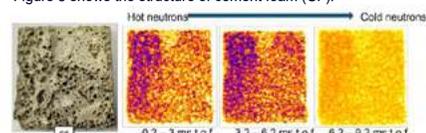


Figure 5: Energy-resolved neutron imaging of CF.

Suprasys (collaboration with industry)

- Development of new equipment for in-situ calorimetry and neutron scattering experiments.

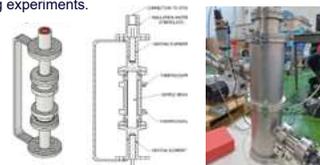


Figure 6. (left) Concept design, (center) technical design and (right) testing and commissioning.

Next steps

- Understand the effects of having different concentrations of portlandite and calcite.
- Obtain information of the structural changes upon deuteration.
- Perform the first measurements with the Suprasys prototype.

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Microscopic insight on cementitious materials using radiation-scattering techniques

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Novel generation of Polymeric Deep Eutectic Solvents Electrolytes for Energy Storage

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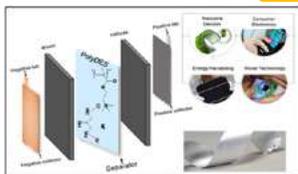
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Why Deep Eutectic Solvents (DES) and PolyDES

- DESs or low melting mixtures are formed only when the intermolecular interactions between two or more components, specifically **hydrogen bond acceptor (HBA)** and **hydrogen bond donor (HBD)**, are stronger than those in individual components. They benefit of **low cost of components, high biobased content and its easy fabrication**.
- Polymerizable deep eutectics known as deep eutectic monomers (DEMs)** are defined as a new class of deep eutectics that contain polymerizable units. DEMs could undergo fast thermal- and/or photo-polymerization to develop polymers (**PolyDES**) having specific features for different applications.



PolyDES in energy-storage application



In the framework of the ADAGIO Fellowship program, **Ion Gel PolyDES Electrolytes (IGPEs)** deriving from DEM mixtures are investigated as quasi all-solid-state electrolytes for energy storage/conversion technologies. The main advantage of IGPEs is the feasibility of combining advantages of both liquid and solid electrolytes simultaneously acting as **flexible, safe, no leaking, green and low-cost separator** in electrochemical devices.

PolyDEM preparation and characterization

- The addition of the polymerizable monomer at different concentration affects the homogeneity of the mixtures
- High monomer amount leads to only partial solubilization
- Dry procedure** (no solvent involved)

DSC analysis showed a single T_g → possible metastable polymorphs

ATR-FTIR spectroscopy to investigate shift due to HHBA-HBD coordination

Application of PolyDES in energy storage devices

Green, Safer, PolyDES Electrolytes for Next Generation Energy Storage devices

Separator Binder

Novel generation of Polymeric Deep Eutectic Solvents Electrolytes for Energy Storage

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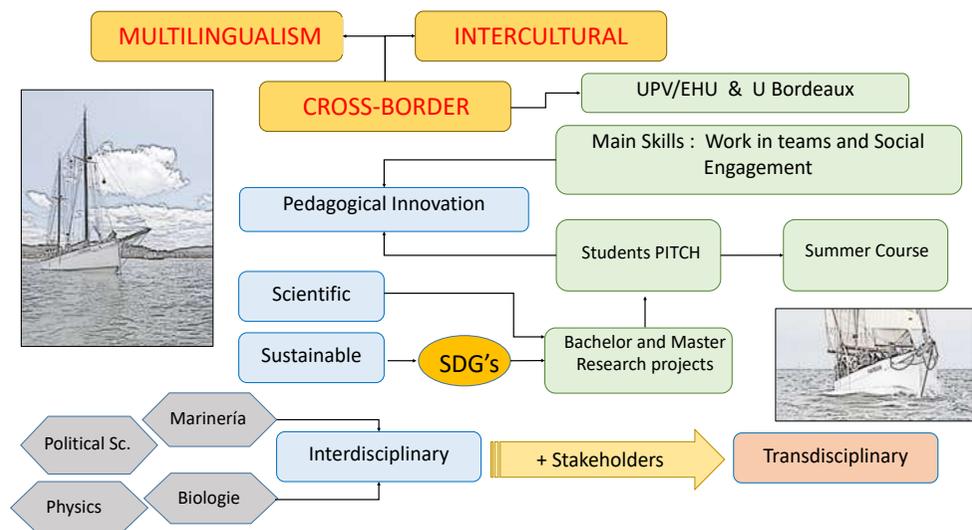
The SALTILLO training ship: cross-border exploration for the health of the marine environment

**Imanol Basterrechea-Iribar UPV/EHU; Manuel Soto PIÉ-UPV/EHU; Mikel Lejarza UPV/EHU Julieta Barrenechea Euskampus Fundazioa*

The 3rd edition of the summer course on board the Saltillo training ship once again brought together students from different disciplines from the UPV/EHU and the UB in a trip that combines learning about seamanship, direct contact with emblematic sites of the Basque coastline and a commitment to transdisciplinary scientific and technical knowledge related to the health of the marine environment. An essential feature of this course is the interculturality and multilingualism that permeate both the coexistence of the crew and the lectures and activities open to the public.

The 2024 edition is a more consolidated version as it has managed to articulate teams and projects that have given it scientific, technical, pedagogical and cross-border and international projection. The design, coordination and execution of this course has been supported by the teams, results, capacities and resources of the Cross-border Cooperation Laboratory -LTC Incubator EAR' of the LTC Sarea Programme, the PIÉ IKD3 Saltillo Educational Innovation Project as a means of facilitating transversal competences and the Euskampus Bordeaux Cross-border Campus of International Excellence.

The course featured lectures by academics and local stakeholders and for the first time, the students crew presented a pitch about their research. With the Saltillo as a meeting point and with the help of institutions, people and projects, the institutional commitment and the transdisciplinary approach needed to address the urgent situation of the marine and coastal environment were strengthened.



The SALTILLO training ship: cross-border exploration for the health of the marine environment

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Whole genome sequencing of multidrug resistant *Acinetobacter baumannii* isolates from a hospital from Paraguay

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BACKGROUND

Acinetobacter baumannii is a pathogen of great clinical importance, capable of causing severe nosocomial infections and acquiring resistance to antimicrobials, specifically carbapenems. Due to the threat to global public health, the World Health Organization classified carbapenem-resistant *A. baumannii* as a priority 1 (critical) pathogen for research and development of new antibiotics. In Latin America, a high rate of carbapenem resistance in *A. baumannii* has been detected, as well as the circulation of some international clones, however, Paraguay is one of the countries with a limited database in this regard. Therefore, the aim of this work was to study multidrug-resistant *Acinetobacter baumannii* clinical isolates from the National Hospital of Itaugua, Paraguay, using molecular biology techniques such as Whole Genome Sequencing (WGS).

MATERIALS AND METHODS

RESULTS

Data analysis from the National Hospital of Itaugua

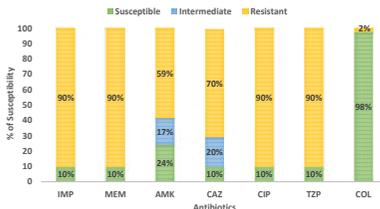


Figure 1. Percentages of antibiotic susceptibility of *A. baumannii* isolates recovered from December 2023 to February 2024. IMP: imipenem, MEM: meropenem, CAZ: ceftazidime, AMK: amikacin, SAM: ampicillin/sulbactam, CIP: ciprofloxacin, GEN: gentamicin.

Resistome and plasmidome analysis of *A. baumannii* isolates from Paraguay

Table 1. Characteristics of the carbapenem resistant isolates from the National Hospital of Itaugua (Paraguay) regarding the clonal lineage, resistance genes and plasmid profile.

Isolate	International Clone (IC)	ST	ST	β -lactamase genes	Other resistance genes	Plasmids (kb)	Replicon typing-PCR	Replicases (Acinetobacter Plasmid Typing Database)
		Pasteur	Oxford					
PR1				<i>bla_{NDM-5}</i> , <i>bla_{OXA-48}</i> , <i>bla_{OXA-24}</i> , <i>bla_{OXA-23}</i> , <i>bla_{OXA-51}</i> , <i>bla_{OXA-52}</i> , <i>bla_{OXA-53}</i> , <i>bla_{OXA-54}</i> , <i>bla_{OXA-55}</i> , <i>bla_{OXA-56}</i> , <i>bla_{OXA-57}</i> , <i>bla_{OXA-58}</i> , <i>bla_{OXA-59}</i> , <i>bla_{OXA-60}</i> , <i>bla_{OXA-61}</i> , <i>bla_{OXA-62}</i> , <i>bla_{OXA-63}</i> , <i>bla_{OXA-64}</i> , <i>bla_{OXA-65}</i> , <i>bla_{OXA-66}</i> , <i>bla_{OXA-67}</i> , <i>bla_{OXA-68}</i> , <i>bla_{OXA-69}</i> , <i>bla_{OXA-70}</i> , <i>bla_{OXA-71}</i> , <i>bla_{OXA-72}</i> , <i>bla_{OXA-73}</i> , <i>bla_{OXA-74}</i> , <i>bla_{OXA-75}</i> , <i>bla_{OXA-76}</i> , <i>bla_{OXA-77}</i> , <i>bla_{OXA-78}</i> , <i>bla_{OXA-79}</i> , <i>bla_{OXA-80}</i> , <i>bla_{OXA-81}</i> , <i>bla_{OXA-82}</i> , <i>bla_{OXA-83}</i> , <i>bla_{OXA-84}</i> , <i>bla_{OXA-85}</i> , <i>bla_{OXA-86}</i> , <i>bla_{OXA-87}</i> , <i>bla_{OXA-88}</i> , <i>bla_{OXA-89}</i> , <i>bla_{OXA-90}</i> , <i>bla_{OXA-91}</i> , 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