

Post-doctoral researcher in nano-ecotoxicology

Title: Interactions of nanoplastics with biological membranes

Institutes: (1) Marine environmental science laboratory (LEMAR) UMR 6539 CNRS/UBO/IRD/Ifremer, Plouzané, France. (2) Institute of Molecules and Materials of Le Mans (IMMM) UMR 6283 CNRS, Le Mans, France.

Gross monthly salary: 2500-3500€ depending on professional experience

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Description of the proposal:

Worldwide annual production of plastics has been steadily increasing since 1950 and was estimated at 311 million tons in 2015¹. Plastics gather more than twenty families of polymers among which polyethylene (PE), polypropylene (PP), polyvinyl chloride (PVC), polyurethane (PUR), polystyrene (PS) and polyamide (PA) are referred as the “big six” as they represent approximately 90% of total world production². After less than a century of existence, plastic debris already represent from 60 to 80 % of the marine litter depending on locations³. Once in the environment, macro debris undergo mechanical (erosion, abrasion), chemical (photo-oxidation, temperature, corrosion) and biological (degradation by microorganisms) actions^{4,5}. Plastic fragmentation is considered to be an endless process and may continue until the molecular level to ultimately lead to the continuous release of microplastics (MP) commonly defined as plastic particles smaller than 5 mm⁶, and even nanoparticles of plastics (NP) in the environment. Here, NP will be defined as plastic particles with all dimensions less than 1 micrometer. Even though it is assumed that can easily cross the biological membranes due to their tiny size, very little is known about their toxicity modes of action.

This project aims to evaluate the interactions between MP/NP and biological membranes of key marine organisms through innovative approaches using membrane vesicles and isolated cells. NP (10-500nm) and small MP (1-20µm) made of PE and PP will be considered. Targeted mollusk bivalve and fish will be the Pacific oyster *Crassostrea gigas* and the European sea bass *Dicentrarchus labrax*, being marine species of ecological and economical interest. For unicellular photosynthetic eukaryotes (UPE) *Rhodomonas salina* and *Chaetoceros neogracile* will be selected in regards with their importance in marine primary production and food source for marine bivalves.

The different nanoparticles will be incubated with (i) membrane vesicles synthesized *in vitro* from mixtures of synthetic lipids in order to build a biomimetic membrane with known composition and mechanical properties; (ii) membrane vesicles prepared using phospholipids and glycolipids extracted from the targeted marine organisms; and (iii) isolated cells. Our aim is to shed some light on the mechanisms involved in the plastics/membrane interaction and identify the possible integration of the NP/small MP in the membrane and their role in the disruption of the membrane architecture. NP behavior will be determined by TEM, as well as their effects on the membrane structural, mechanical and functional properties. Changes in membrane lipid composition will be measured by combining HPLC (polar lipid class separation) and GC-FID (Fatty acid composition of lipid classes)^{7,8}. Nuclear Magnetic Resonance (NMR), Raman microscopy, Atomic force microscopy (AFM) and fluorescent

probe molecules of the membrane core and surface will be used in order to assess the membrane properties after exposition to NP.

Impacts of MP/NP on UPE will be measured through the assessment of specific growth rate, morphological information of cell size and complexity, cell chlorophyll content, cell viability, intracellular lipid content and metabolic activity using a flow cytometer following protocols previously developed in the laboratory⁹. For animal cells, the effects of MP/NP on various membrane dependent processes/responses will be investigated. This includes phagocytosis, lysosomal stability, membrane integrity, apoptosis, and mitochondrial membrane potential for bivalve hemocytes and fish neutrophil granulocytes; B cell proliferation and IgM secretion for fish lymphocytes; and chloroplast and membrane integrity for UPE. All these membrane-related measurements will be performed using flow cytometry as already described^{10,11,12,13}.

Requirements (training/expertise) and profile: The candidate will be required to have a strong expertise in analytical chemistry and lipid biochemistry. Excellent knowledge of lipidomic techniques such as liquid chromatography and mass spectrometry. Basic knowledge about biology, membrane biophysics or spectral analysis techniques would be beneficial. The candidate will have to conduct the experiments, analyze the results, including statistical analyses (e.g. by ANOVA) and write up in the form of high quality reports and scientific manuscripts for publication. Presentations to various audiences including scientists, policy makers and the general public will also be expected. Travels and exchanges between the two institutes will be required.

The candidate must have spent at least 12 months outside of France over the last three years from the request for proposal opening date (May 2015). To apply, submit a cover letter indicating past research experience, motivation for the position, expected availability date and curriculum vitae directly to ika.paulpont@univ-brest.fr.

Bibliography:

- 1 PlasticsEurope, 2015
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- 6 National Oceanic and Atmospheric Administration (NOAA) Proceedings of the International Research Workshop on the Occurrence, Effects, and Fate of Microplastic Marine Debris, C. Arthur, J. Baker, and H. Bamford (eds.), Technical Memorandum NOS-OR&R-30, September 9-11, 2008, University of Washington Tacoma, Tacoma, WA, USA.
- 7 Le Grand F. et al. (2013) *Chem phys lipids*, 167, 9-20.
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- 9 Lelong A. et al. (2012) *Aquatic Toxicology* 118, 37-47.
- 10 Hégaret H. et al. (2011) *Cell biol toxicol*, 27 (4), 249-266.
- 11 Danion M., et al. (2011) *Aquat Toxicol*, 105, 300-311.
- 12 Donaghy L. et al. (2012) *PloS one* 7 (10), e46594.
- 13 Martins K., et al. (2015) *Fish & Shellfish Immunology*, 44, 332-341.