



NEW FLUORESCENT PROTEINS FROM THE SEA

Thesis submitted for the degree of Doctor in Philosophy (Ph.D.) in
Life, Health and Chemical Sciences.

Marie-Lyne MACEL

Affiliated Research Centre (ARC):

Stazione Zoologica Anton Dohrn, Naples

The Open University

September 2020

Director of Studies: Dr Salvatore D'Aniello, Biology and Evolution of
Marine Organisms, Stazione Zoologica Anton Dohrn, Naples

Internal Supervisor: Dr Annamaria Locascio, Biology and Evolution
of Marine Organisms, Stazione Zoologica Anton Dohrn, Naples

External Supervisor: Dr Daniela Corda, Institute of Biochemistry,
CNR, Naples

ABSTRACT

The focus of this PhD project is to find a new fluorescent protein (FP) with spectral properties different from the ones already discovered, mainly in the red, far-red part of the spectrum. In this region, less light is scattered, absorbed and re-emitted by endogenous biomolecules, which makes it particularly interesting.

The green fluorescent protein (GFP) being the first FP isolated in the hydrozoa *Aequoria victoria*, many other GFPs were isolated in this clade as well as in Anthozoa, Arthropoda and Cephalochordata. Also, new FPs have been characterized in Vertebrata, namely UnaG and Sandercyanin, with features completely different from GFPs; UnaG being a fatty-acid-binding protein and Sandercyanin a lipocalin.

This work has been undertaken by several means; Long Term Ecological Research station (LTER-MareChiara) sampling in the gulf of Naples by assessing the epifluorescent and spectral properties of zooplanktons and thanks to bioinformatics tools exploring TARA database.

Many clades of zooplanktons have been examined throughout the PhD project with a focus on Annelid also with the sampling that was carried out on the seaside. The spectral properties of the zooplanktons were assessed scrupulously by confocal microscopy, as a test response to chlorophyll was able to rule out positive organisms and a test to UV excitation and visible light spectra allowed identifying species with red, far-red fluorescent emission patterns.

The exploration of available transcriptomes, genomes and TARA eukaryotic database by blastp search has been performed using different classes of reference proteins UnaG, Sandercyanin and 14 GFPs from four different groups. No similarity from the protein alignments were found with UnaG and Sandercyanin whereas the alignments resulted in the findings of GFP chromophores in 25 hits sequences from which we produced a phylogenetic tree.