# Ecole Doctorale COMPLEXITE DU VIVANT – Fiche Projet CONCOURS

***Fiche à nommer selon le format Nom_Prenom (sans accents ni cédilles), à enregistrer en format PDF et à renvoyer à l'adresse : edcdv@sorbonne-universite.fr***

**Nom et prénom du directeur de thèse (et si besoin du co-directeur) : CROCE Jenifer**
*Le directeur de thèse et le co-directeur doivent impérativement avoir l'HDR ou équivalent*

Coordonnées :
Evolution of Intercellular Signaling in Development (EvoInSiDe) Team
Laboratoire de Biologie du Développement de Villefranche-sur-Mer (LBDV) (UMR7009 - CNRS/SU)
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**Y-a-t-il un candidat déjà identifié pour le projet :** OUI ~~NON~~

**Nom et prénom du responsable de l'équipe : CROCE Jenifer & SCHUBERT Michael**

**Intitulé de l'équipe : Evolution of Intercellular Signaling in Development (EvoInSiDe)**

**Nombre de chercheurs et enseignants-chercheurs statutaires de l'équipe titulaires d'une HDR (ou équivalent) : 2**

**Nom et prénom du responsable d'UMR ou de département : McDOUGALL Alex**

**Intitulé et N° d'UMR ou de département : Laboratoire de Biologie du Développement de Villefranche-sur-Mer (UMR7009)**

**Titre du projet de thèse** : **Characterization of the developing adult nervous system of the sea urchin *Paracentrotus lividus***

**Signature du directeur d'UMR ou de département (vaut avis favorable pour le dépôt du projet) :**

**Spécialité : Developmental Biology**

**Résumé du projet de thèse** *(1 page maximum, en anglais)*
*Pour les thèses avec 2 co-directeurs, ou en partenariat entre 2 laboratoires ou structures, indiquer la participation de chaque co-directeur et structure dans la gestion du projet.*

Throughout the animal kingdom, the function of a nervous system, is to collect, process, and respond to sensory inputs from the environment and the body. To date, although enormous advances have been made in our understanding of their physiology, neurobiology, development, and cell biology, the evolutionary history of metazoan nervous systems remains highly contentious[1-3]. The current consensus in the scientific community is that the key to resolving this question is to study and characterize the nervous systems of so-called 'minor/non-classical' animal lineages. One such 'minor/non-classical' lineage, which has so far been largely neglected in comparative analyses of adult nervous systems, is that of the echinoderms.

The echinoderms include five different animal classes: the echinoids (or sea urchins), the asteroids (or sea stars), the ophiuroids, the holothuroids (or sea cucumber), and the crinoids[4]. Although species from most of these classes have already been used, through the past decades, as developmental biology models to study processes such as fertilization, cell division, gastrulation movements, or larval nervous system development, studies examining the development of their adult structures remain scarce. This is chiefly due to the pentaradial symmetry of their adult bodies as well as the difficulty of rearing them in the laboratory through the larval period and post-metamorphosis[5,6]. Recently, in the team, we have however been able to carry out pilot studies on the characterization of the development of the adult nervous system of three echinoderm species, from three different echinoderm classes (a sea urchin, a sea cucumber, and a sea star)[7]. We have thereby compiled detailed anatomical descriptions of the adult nervous system of these three species and have demonstrated that they are largely similar[7]. We have also recently acquired, for the sea urchin, high-throughput sequencing-based transcriptomes, by RNAseq analyses, at different stages of adult nervous system development.

The goal of this project is to build on these anatomical and transcriptomic datasets to carry out the first molecular and functional characterization of the development of the adult nervous system of an echinoderm, using as a model species the sea urchin *Paracentrotus lividus*. More specifically, the project is subdivided into two tasks, which aim at addressing specifically the molecular and gene regulatory network aspects of *P. lividus* developing adult nervous system:
- *Task 1. Establish a detailed molecular map of the developing adult nervous system of the sea urchin* P. lividus. This task will be based on the transcriptome datasets that are already available, which will be coupled to *in situ* hybridization assays, on *in toto* specimens, carried out in multicolor, to establish co-expression of specific gene cohorts in the same tissues. Our investigation will be directed chiefly to transcription factors, signaling molecules, and neurotransmitter-related genes presenting differential expression profiles in our transcriptome datasets. Of these, our focus will moreover be first on genes reported as involved in sea urchin larval and metazoan nervous systems establishment.
- *Task 2. Determine the molecular networks involved in regulating the location, specification, patterning, and differentiation of the adult nervous system of the sea urchin* P. lividus. This task will be based, initially, on pharmacological treatments to inhibit or stimulate the activity of intercellular signaling pathways selected on the basis of differential expression analyses of our transcriptome datasets. The treatments will be conducted starting at different developmental stages as well as carried out through distinct time periods, to identify all of the possible roles of the pathways during sea urchin adult nervous system development. Then, gene-specific loss-of-function assays will be conducted on genes related to the studied intercellular signaling pathways, to corroborate the results obtained through pharmacological treatments, as well as on transcription factors selected from Task 1 to identify the genetic processes underlying sea urchin adult nervous system development. This approach will be based on the use of a stage-specific inducible technique we recently developed in the team for *P. lividus*.

Taken together, the proposed project will generate the first molecular and functional characterization of the development of the adult nervous system of an echinoderm, including molecular signatures of developing neural tissues and the first insights into the gene regulatory networks underlying neural development. We plan to use the data obtained in the course of this project for detailed comparisons with adult nervous systems from species of other echinoderm classes as well as from species of other animal lineages. Our long-term goal is to add substantial experimental evidence to the ongoing discussion about the evolution of animal nervous systems, at least in bilaterians.

References:

1. Arendt, D., *et al*. (2016) From nerve net to nerve ring, nerve cord and brain - evolution of the nervous system. *Nature Reviews Neuroscience* 17, 61–72.
2. Martín-Durán, J.M., *et al*. (2018) Convergent evolution of bilaterian nerve cords. *Nature* 553, 45–50.
3. Formery, L., *et al.* (2019) Ambulacrarians and the ancestry of deuterostome nervous systems. *Results and Problems inCell Differentiation* 68, 31–59.

4. Telford, M.J., et al. (2014) Phylogenomic analysis of echinoderm class relationships supports Asterozoa. *Proceedings of the Royal Society B: Biological Sciences* 281, 20140479.
5. Holland, L.Z. (2015) Evolution of basal deuterostome nervous systems. *Journal of Experimental Biology* 218, 637–645.
6. Benito-Gutiérrez, E. and Arendt, D. (2009) CNS evolution: new insight from the mud. *Current Biology* 19, R640–R642.
7. Formery, *et al.* (2021) Neural anatomy of echinoid early juveniles and comparison of nervous system organization in echinoderms. *Journal of Comparative Neurology* 529, 1135–1156.

**Faisabilité du projet de thèse (*1/2 page maximum, en anglais)***

*Expliciter la faisabilité du projet en terme d'expertise de l'équipe d'accueil, des collaborations potentielles qui pourront être mises en place pour certains aspects du projet, de la disponibilité des appareils nécessaires au bon déroulement du projet...*

The proposed project will be carried out in the Evolution of Intercellular Signaling in Development (EvoInSiDe) team at the Laboratoire de Biologie du Développement de Villefranche-sur-Mer (LBDV) of the Institut de la Mer de Villefranche (IMEV). The members of the EvoInSiDe team have been studying the early development of sea urchins for more than 20 years[1-3] and, more recently, the post-metamorphic development of sea urchins[4]. The proposed project will thus benefit from established protocols along with preliminary data. As examples, the team has already developed an efficient husbandry system for the sea urchin species *P. lividus*, which allows the growth of large numbers of larvae through metamorphosis[4]. The team has further established protocols for single and double, chromogenic or fluorescent, *in situ* hybridization, in both *P. lividus* larvae and juveniles, *in toto* or cryo-sectioned[3,4]. The team also has access to a large transcriptome dataset obtained by RNAseq analyses carried out at different stages of adult nervous system development. Finally, the team has extensive experience with pharmacological treatments and functional analysis of developing *P. lividus*[1-3] and has just validated a protocol for stage-specific gene knockdown in *P. lividus*, which has already been applied successfully to late embryos and larvae. In addition, the project will benefit from informatic and administrative support at the level of the LBDV as well as from animal and microscopy platforms at the level of the IMEV. Taken together, based on the available knowhow, resources, and support, we are confident that the risks of the proposed project are manageable and that all the proposed tasks are feasible.

References:

1. Croce, J.C., et al. (2003). Coquillette, a sea urchin T-box gene of the Tbx2 subfamily, is expressed asymmetrically along the oral–aboral axis of the embryo and is involved in skeletogenesis. *Mechanisms of Development*, 120, 561–572.
2. Croce, J.C., et al. (2006) Frizzled5/8 is required in secondary mesenchyme cells to initiate archenteron invagination during sea urchin development. *Development* 133, 547–557.
3. Lhomond, G., et al. (2012) Frizzled1/2/7 signaling directs β-catenin nuclearisation and initiates endoderm specification in macromeres during sea urchin embryogenesis. *Development* 139, 816–825.
4. Formery, L., et al. (2021) Neural anatomy of echinoid early juveniles and comparison of nervous system organization in echinoderms. *Journal of Comparative Neurology* 529, 1135–1156.

**Thèses actuellement en cours dans l'équipe**

*Tous les encadrements doivent être indiqués (y compris les co-directions avec un autre HDR pour des doctorants d'une autre ED, et les encadrements dans le cadre de programmes doctoraux tels qu'IPV, FDV...)*

| Nom et Prénom du doctorant | Directeur(s) de thèse | Année de 1ère inscription | ED | Financement |
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## Ecole Doctorale COMPLEXITE DU VIVANT – Fiche Projet CONCOURS

**Trois publications récentes du directeur de thèse** (du co-directeur ou du co-encadrant s'il y a lieu). Mettre en gras le nom du directeur de thèse.

Formery, L., Orange, F., Formery, A., Yaguchi, S., Lowe, C. J., Schubert, M. and **Croce, J. C**. (2021). Neural anatomy of echinoid early juveniles and comparison of nervous system organization in echinoderms. *Journal of Comparative Neurology* 529, 1135–1156.

Formery, L., Schubert, M. and **Croce, J. C**. (2019). Ambulacrarians and the ancestry of deuterostome nervous systems. *Results and Problems in Cell Differentiation* 68, 31–59.

Zieger, E., Candiani, S., Garbarino, G., **Croce, J. C.,** and Schubert, M. (2018). Roles of retinoic acid signaling in shaping the neuronal architecture of the developing amphioxus nervous system. *Molecular Neurobiology* 55, 5210–5229.

**Docteurs encadrés par le directeur de thèse** ayant soutenu entre la date de dépôt de ce dossier et il y a 5 ans et publications relatives à leur sujet de thèse. Mettre en gras le nom du directeur de thèse et celui du docteur.

Nom Prénom : FORMERY, Laurent

Date de soutenance : 11 octobre 2019
Durée de thèse (en mois) : 48 mois
Ecole Doctorale : ED515

Publications :

- **Formery, L.**, Wakefield, A., Gesson, M., Toisoul, L., Lhomond, G., Gilletta, L., Lasbleiz, R., Schubert, M., **Croce, J.C.** (in prep., to be submitted to Frontiers in Cell and Developmental Biology special issue) Developmental atlas of the indirect developing sea urchin *Paracentrotus lividus*: from fertilization to juvenile stages.

- **Formery, L.,** Orange, F., Formery, A., Yaguchi, S., Lowe, C.J., Schubert, M., **Croce, J.C.** (2021) Neural anatomy of echinoid early juveniles and comparison of nervous system organization in echinoderms. *Journal of Comparative Neurology* 529, 1135–1156.

- **Formery, L.**, Schubert, M., **Croce, J.C.** (2019) Ambulacrarians and the ancestry of deuterostome nervous systems. *Results and Problems in Cell Differentiation* 68, 31–59.

- Cosse-Etchepare, C., Gervi, I., Buisson, I., **Formery, L.,** Schubert, M., Riou, J.-F., Umbhauer, M., Bouffant, R.L. (2018) *Pou3f* transcription factor expression during embryonic development highlights distinct *pou3f3* and *pou3f4* localization in the *Xenopus laevis* kidney. *International Journal of Developmental Biology* 62, 325–333.