Master Biologie Moléculaire et Cellulaire 'BMC',
Université de Paris - *UFR Sciences du Vivant*

Parcours : **Biologie et Développement Cellulaires 'BDC'**
http://www.master2bdc.fr/
Fiche de Projet de Stage M2, Année 2021-2022

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| **Unité INSERM ou CNRS ou Université :**<br><br>**Intitulé Equipe :**<br>**Microtubule Regulation in Multi-cellular Animals**<br><br>**ED d'appartenance :**<br>**BioSPC**<br><br>**Responsable de l'Equipe :**<br>**Paul Conduit** | **Responsable du Stage : Paul Conduit**<br><br>**Contacts**<br>Adresse : Institut Jacques Monod, CNRS - Université de Paris, 15 rue Hélène Brion, 75013 Paris<br><br>Email : paul.conduit@ijm.fr<br><br>Tel : 01 57 27 80 95 |

**Titre du projet : Using single-molecule TIRF imaging to study microtubule nucleation**

**Résumé du Projet de Stage** (en 300 mots maximum, mots clés en gras)
Microtubules are nucleated by multi-protein γ-tubulin ring complexes (γ-TuRCs). These complexes are recruited and activated at microtubule organising centres (MTOCs) within cells. The mechanism of activation is currently unclear, but is thought to involve structural rearrangements of the γ-TuRC. One of the key modes of γ-TuRC recruitment to MTOCs is via binding to tethering proteins that contain a CM1-domain, such as *Drosophila* Centrosomin (Cnn). Binding of these tethering proteins to γ-TuRCs appears to activate γ-TuRCs, thus linking recruitment and activation, but the precise mechanism is yet to be determined.

The M2 student will purify GFP-tagged γ-TuRCs from *Drosophila* embryos (using a method already established in the lab) and then use single-molecule TIRF imaging to perform *in vitro* microtubule nucleation assays in order to test γ-TuRC activity (microtubule nucleation from individual γ-TuRCs can be monitored). The student will test whether adding purified fragments of Cnn, or other factors such as kinases, increases the nucleation efficiency of γ-TuRCs. The student will also test an alternative model: that Cnn binding stimulates the assembly of non-canonical active γ-TuRCs, rather than activating pre-formed γ-TuRCs. The student's results will be used, in conjunction with cryo-EM structural assays by our collaborators, to establish whether Cnn binding can induce structural modifications in γ-TuRCs.

The student will be encouraged to be proactive and help develop methods for performing the single-molecule imaging, coming up with ideas and taking initiative. Alongside the more complex techniques, the student will also learn the basics of *Drosophila* husbandry in order to collect the embryos used for γ-TuRC purification. The student will have the possibility to return as a PhD student.

**Publications de l'équipe relatives au projet de stage (max 5)**
Tovey T, Tsuji C, Egerton A, Bernard F, Guichet A, Roche MA, Conduit PT*. Auto-inhibition of Cnn binding to γ-TuRCs prevents ectopic microtubule nucleation and cell division defects. BioRxiv. doi: https://doi.org/10.1101/2020.10.05.326587

Mukherjee A, Brooks P, Bernard F, Guichet A, Conduit PT*. (2020). Microtubules originate asymmetrically at the somatic Golg and are guided via Kinesin2 to maintain polarity in neurons. eLife. DOI: 10.7554/eLife.58943

Tovey CA, Tubman CE, Hamrud E, Zhu Z, Dyas AE, Butterfield AN, Fyfe A, Johnson E, Conduit PT*. (2018). γ-TuRC heterogeneity revealed by analysis of Mozart1. Current Biology 28, 2314-2323.

Tovey CA and Conduit PT*. (2018). Microtubule nucleation by γ-tubulin complexes and beyond. Essays in Biochemistry. DOI: 10.1042/EBC20180028

Feng Z, Caballe A, Wainman A, Johnson S, Haensele AFM, Cottee MA, Conduit PT, Susan M. Lea, Jordan W. Raff. (2017). Structural Basis for Mitotic Centrosome Assembly in Flies. Cell 169, 1078–1089.