**Proposition de sujet de thèse**

**Campagne 2017 d’attribution des contrats doctoraux attribués à EDCSV**

**Directeur de thèse** (Supervisor), HDR rattaché à EDCSV:

*Irina Gutsche, IBS, HDR 2012*

**Co-Directeur de thèse** (éventuel), titulaire HDR :

*Dominique Bourgeois, IBS, HDR 1999*

**Unité de Recherche**/Laboratory :

*Institut de Biologie Structurale, Directeur Prof. Winfried Weissenhorn*

**Equipe de recherche**/Research team :

*Molecular Machines in Bacteria and Viruses (Irina Gutsche), part of the MEM group (Guy Schoehn)*

*Pixel (Dominique Bourgeois), part of the DYNAMOP group (Martin Weik)*

**Titre du projet de thèse** (en français): Cryo-tomographie électronique et microscopie de super-résolution des minicellules bactériennes

**Titre du projet de thèse** (en anglais): Cryo-electron tomography and super-resolution microscopy of bacterial minicells

**Résumé** (en anglais): Précisez le contexte, les objectifs, les méthodes (500 mots maximum)

Cryo-electron microscopy (cryo-EM), cryo-electron tomography (cryo-ET) and super-resolution microscopy (SRM) are currently revolutionizing structural biology. However, one of the main reasons why until now only a handful of bacterial proteins have been visualized in whole cells by cryo-ET is the thickness of the specimen. Indeed, an *E. coli* cell is ~1 µm thick, whereas the thickest biological specimen that can be studied directly by cryo-EM should be thinner than 0.5 µm. A novel way to overcome this limitation is to work with minicells, in which the cell division septum is aberrantly placed adjacent to the cell pole instead of in the cell middle, resulting in abnormal cell division producing small (100-500 nm) cells that contain all molecular components of the parent cell except the chromosome. These cells, capable of protein synthesis from ectopic DNA and normal metabolic functions, are ideally sized for cryo-ET and we think that they may provide a revolutionary tool to solve the structure of large macromolecular assemblies directly *in cellulo*. However, locating such assemblies, potentially expressed at very low copy number, within the crowded cellular environment poses a challenge. To solve this challenge, correlative approaches combining SRM and ET currently offer great promises. The present project aims to use minicells expressing nanocages of controlled size as a tool to develop super-resolution cryo-CLEM (cryogenic correlative light and electron microscopy). We already observed several types of available minicells by cryo-EM and engineered nanocages-expressing minicell strains. The objectives of this PhD project are to (i) optimize the current minicell preparation, (ii) collect cryo-ET datasets on the currently best microscope, the Titan Krios with an energy filter, a direct electron detector and a phase plate, (iii) collect, at cryogenic temperatures, super-resolution PALM data sets of nanocages labeled with photoactivatable fluorescent proteins, in order to precisely locate them within the minicells, (iv) compare this cryo-PALM-based localisation with computational approaches relying on template matching in the reconstructed cryo-tomograms, (v) solve the structure of the located nanocages by subtomogram averaging, (vi) compare those structures with cryo-EM structures obtained *in vitro* from purified components. This very ambitious project is designed to open up a new area of research since we expect that the methods developed here can be extended, adapted and implemented for a variety of highly functionally relevant biological systems.

**Mots-clés** (5 maximum) : en anglais et en français

Cryo-electron microscopy, subtomogram averaging, cryogenic super-resolution fluorescence microscopy, cryo-correlative microscopyMicroscopie cryo-electronique, alignement des sous-tomograms, microscopie de fluorescence super-resolution a temperature cryogenique, microscopie cryo-correlative

**Sujet éligible à une allocation de la Fondation pour la recherche médicale (FRM) :**

Oui **□** Non **X**

**Profil du candidat souhaité :**

We are looking for a student interested in structural biology, ideally with a physics or chemistry background and with a strong interest in microscopy, method development and computing. Some experience in working in a Linux environment and programming would be an advantage. The student will work in a highly interdisciplinary and international environment and should be willing to collect data at leading cryo-EM facilities throughout the world.

**Trois publications récentes du Directeur de thèse** (et du co-directeur, s’il y a lieu) :

Kandiah E, Carriel D, Perard J, Malet H, Bacia M, Liu K, Chan SW, Houry WA, Ollagnier de Choudens S, Elsen S, Gutsche I.

“Structural insights into the Escherichia coli lysine decarboxylases and molecular determinants of interaction with the AAA+ ATPase RavA.”

*Sci Rep.* 2016 Apr 15;6:24601. doi: 10.1038/srep24601.

Gutsche I, Desfosses A, Effantin G, Ling WL, Haupt M, Ruigrok RW, Sachse C, Schoehn G.

 “Near-atomic cryo-EM structure of the helical measles virus nucleocapsid.”

*Science.* 2015 May 8;348(6235):704-7. doi: 10.1126/science.aaa5137.

Malet H, Liu K, El Bakkouri M, Chan SW, Effantin G, Bacia M, Houry WA, Gutsche I.

“Assembly principles of a unique cage formed by hexameric and decameric E. coli proteins.”

*Elife.* 2014 Aug 5;3:e03653. doi: 10.7554/eLife.03653.

R. Berardozzi, V. Adam, A. Martins and D. Bourgeois “Arginine 66 controls dark-state formation in green-to-red photoconvertible fluorescent proteins”
*J. Am. Chem. Soc.*, (2016) 138 (2), 558–565 DOI: 10.1021/jacs.5b09923

Jacq M, Adam V, Bourgeois D, Moriscot C, Di Guilmi A-M, Vernet T, Morlot C.  “Remodeling of the Z-ring nanostructure during the Streptococcus pneumoniae cell cycle revealed by photoactivated localization microscopy”
*mBio*, (2015) 6(4):e01108-15. doi:10.1128/mBio.01108-15

V. Adam, R. Berardozzi, M. Byrdin and D. Bourgeois
“Phototransformable fluorescent proteins: Future challenges”
*Curr. Opin. Chem. Biol.*, (2014), 92-102.

**Docteurs encadrés par le Directeur de thèse** (et du co-directeur s’il y a lieu) **ayant soutenu leur thèse** (dans les 5 dernières années). Indiquer la date de soutenance, la durée de la thèse (en mois), les publications relatives au sujet de thèse et leur situation actuelle :

Romain Berardozzi : 3 décembre 2016 / 38 mois / 4 publications / préparateur agrégé
Duan Chenxi : 5 décembre 2014 / 38 mois / 3 publications / presse scientifique

**Thèses en cours encadrées par le Directeur de** **thèse** (et du co-directeur s’il y a lieu, dupliquer le tableau)

Irina Gutsche :

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| --- | --- | --- | --- |
| Nom, Prénom du doctorant | Date de début de thèse | Type de financement | Indiquer, le cas échéant, s’il s’agit d’une co-direction, d’une co-tutelle,… |
| Diego Carriel Lopez | 01/10/2014 | ANR | Co-direction (soutenance programmée pour le 14/04/2017) |
| Matthew Jessop | 01/10/2017 | IRTELIS |  |

Dominique Bourgeois :

|  |  |  |  |
| --- | --- | --- | --- |
| Nom, Prénom du doctorant | Date de début de thèse | Type de financement | Indiquer, le cas échéant, s’il s’agit d’une co-direction, d’une co-tutelle,… |
| Daniel Thédie | 15/10/2016 | IRTELIS |  |
| Kevin Floch | 01/10/2015 | IRTELIS | Co-direction |

**Nombre de chercheurs et enseignant-chercheurs titulaires d’une HDR dans l’équipe** : 6 dans les deux groupes

**Nombre total de thèses en cours dans l’équipe :** 6 dans les deux groupes (dont une qui doit etre soutenue le 14/04/2017)