Submission date	**30/09/2022**
Each year deadline	**September 30**

Acronym of the R&T project (max 10 characters)

Patho-XLMS

Title of the R&T project (max 100 characters including spaces)

Deciphering host-pathogen interactions with *in vivo* cross-linking mass spectrometry (XL-MS)

Summary of the R&T project (max 500 characters including spaces)

The ambition of the project is to dissect host-pathogen interactions at the molecular level of proteins using innovative *in vivo* cross-linking mass spectrometry approaches. Infections with the Gram-negative bacterium *Neisseria meningitidis* will be first explored focusing on unresolved issues related to adhesion of the bacteria to host cells. The methods developed will be broadly applicable to other pathogens, bacterial, viral or fungal.

Co-Director Supervisor 1 (main hosting structure: decision maker)

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Co-Director Supervisor 2

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R&T project (max 2,500 characters including spaces)

As illustrated by the current Covid-19 pandemic, infectious diseases remain a major challenge for humanity. Understanding key mechanisms of diseases represent the first and necessary step towards the development of either vaccines or treatments. One critical step of the viral, fungal or bacterial infections is the interaction between the pathogen and its target host cells. Dissecting this interaction at the molecular level and thus at the protein level is therefore of the utmost importance.

Cross-linking mass spectrometry (XL-MS) has recently evolved toward *in vivo* applications to achieve the characterization of protein-protein interactions in live cells on a system-wide scale. However, none of the developed workflows has proved efficient enough to tackle the

challenge of studying host-pathogen interactions. Indeed, in this case, the complexity of the peptide mixture is dramatically increased by the presence of two distinct organisms in the samples (host and pathogen) raising the technological challenge to a new limit. The ambition of our project is therefore to push *in vivo* XL-MS to an unprecedented level of sensitivity to dissect host-pathogen interactions at the molecular level. The ultimate goal is to obtain a full description of protein-protein interactions taking place during the different steps of the infection with a resolution at the structural level thanks to the spatial information brought by the cross-linker length. The data obtained will provide an unprecedented source of information on physiological changes experienced by both host cells and pathogen during infection as well as an exhaustive list of important protein interactions.
To achieve this goal, a completely new class of smart "self-immolable" cross-linkers has been synthesized by organic chemists in the framework of an ANR-funded project. They will be used in conjunction with dedicated LC-MS/MS and data mining methods. The sepsis and meningitis causing bacterial pathogen *Neisseria meningitidis* will be explored with our optimized XL-MS approach to demonstrate its efficiency and address important biological questions. Key steps of interactions of this pathogen with host cells remain unclear. First and foremost, the nature of adhesion receptor(s) of this deadly pathogen is still not fully established.
Importantly, our approach will be possibly extended to any infection and pathogens of different origins, bacteria, virus or parasites**.**

R&T added value of the co-direction of this project (max 1,000 characters including spaces)

This multidisciplinary project will take place between the research unit headed by G. Duménil (GD), expert in *N. meningitidis* infections, and the UTechS headed by J. Chamot-Rooke (JCR) expert in advanced mass spectrometry. Both teams have a long experience of working together, including co-direction of students, and producing groundbreaking work. For this project, the PhD student will be co-supervised by GD and JCR. He/she will share his/her time between labs and will be trained by both, which will be facilitated by their proximity (same building at the IP). The PhD student will have access to all available equipment and will participate in the weekly lab meetings of each team. In addition to regular 1:1 meetings organized with each supervisor, a monthly meeting including both will be scheduled to evaluate the progress of the PhD study programme in terms of the research project, but also training activities, participation to conferences and publications. The double competence acquired by the PhD student in biology and state-of-the-art technology will be a clear asset for his/her future career.

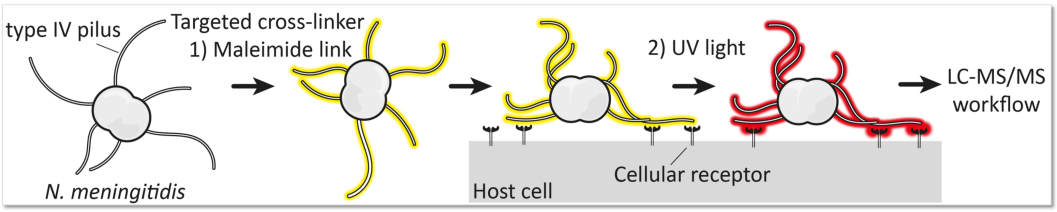
Organisation of the project among partners (work packages, roles and communication)

WP1: Global interactome of *N. meningitidis* infection of endothelial cells (MSBio UTechS)

This WP is based on new cross-linking reagents derived from NNP9 [1]. NNP9 is a trifunctional cross-linker that primarily targets lysines thanks to two NHS functions. It also carries an azido group for an efficient enrichment of cross-linked peptides through click-chemistry. NNP9 has already shown a great potential for *in vivo* XL-MS experiments [2] and several derivatives targeting other amino acids (Asp, Glu) have already been synthesized. However, dead-ends formed by the hydrolysis of one NHS group still represent the majority of the peptides identified, which clearly hinders an in-depth analysis. The new generation of cross-linkers we have entirely designed is "self-immolable" [3] and will allow a complete removal of dead-ends before the MS analysis and thus a huge leap forward in the quality of the data obtained. These reagents are synthesized by the COBRA lab (Rouen) headed by PY Renard in the framework of a ANR funded project. The optimization of all XL-MS steps will be primarily done on BSA, which is the model protein used as a gold-standard in XL-MS experiments, then on intact *N. meningitidis* cells and finally on bacteria in interaction with HUVEC endothelial cells. Bacterial cells will first be placed in contact with host cells and the cross-linking reaction will be done. Bacteria adhere to the cellular surface and rapidly proliferate to form large aggregates that can typically contain 50-100 individual bacteria 2-hours post-infection. With the appropriate multiplicity of infection, most endothelial cells are infected ensuring important quantities of potential cross-links. Data analysis will be performed using Mass Spec Studio, which is developed by the Schriemer's group with whom the MSBio UTechS has a collaboration.

WP2: Interactome of bacterial Type IV pili at the cell surface (UPIV Unit)

As a complement to the untargeted large-scale approach of WP1, we will develop a targeted one to capture the human binding partners of *N. meningitidis* type IV pili, the main adhesive structure expressed by this bacterium. The first step will be to label the cysteine residue previously introduced in the *N. meningitidis* pilin (T130C) in live bacteria with a dedicated cross-linker containing a maleimide and a photoactivable diazirine group [4]. Once this step is validated by regular MS-based proteomics, the bacterial cells carrying the cross-linker will be placed in contact with endothelial cells (HUVECs) and UV light will be used to induce the cross-linking thanks to the presence of the diazirine group. Once the cross-linking step is completed, proteins will be trypsin digested and the cross-linked peptides will be clicked on photocleavable beads for efficient contaminants removal. The sample will be analyzed by LC-MS/MS and cross-linked peptides (and thus target receptor) identified with Mass Spec Studio. As preliminary result, the feasibility of a single cysteine mutation and maleimide cross-linking has already been checked.



J. Chamot-Rooke will supervise all MS experiments and G. Duménil experiments related to infection. The PhD candidate will share his time between the labs which are conveniently located in the same building. A monthly meeting will be organized to discuss results.

[1] Rey *et al. Anal Chem* 90, 10107 (2018)
[2] Rey *et al. Anal Chem* 93, 4166 (2021)
[3] Gonzaga *et al. J Pharm Sci* 109, 3262 (2020)
[4] Ellison *et al. Science* 358, 535 (2017)

R&T candidate background required for developing the project

We will look for an ambitious young scientist motivated to address key biological questions in the field of infections by advanced technologies that can be used for broad applications. Ideally the candidate (biologist, biochemist, chemist) will have a preliminary practical experience in microbiology and/or mass spectrometry.

Selected publications or patents (max 5) of partners

1. Advanced *In Vivo* Cross-Linking Mass Spectrometry Platform to Characterize Proteome-Wide Protein Interactions, Rey M, Dhenin J, Kong Y, Nouchikian L, Filella I, Duchateau M, Dupré M, Pellarin R, **Duménil G**, **Chamot-Rooke J**, *Anal. Chem.* 93, 4166–4174 (2021).
   DOI: 10.1021/acs.analchem.0c04430

2. EXL-MS: An Enhanced Cross-Linking Mass Spectrometry Workflow to Study Protein Complexes, Rey M, Dupré M, Lopez-Neira I, Duchateau M, **Chamot-Rooke J**, *Anal Chem.* 90, 10707-10714 (2018).
   DOI: 10.1021/acs.analchem.8b00737

3. Posttranslational Modification of Pili upon Cell Contact Triggers N. meningitidis Dissemination. **Chamot-Rooke J**, Mikaty G, Malosse C, Soyer M, Dumont A, Gault J, Imhaus AF, Martin P, Trellet M, Clary G, Chafey P, CamoinL, Nilges M, Nassif X, **Duménil G**, *Science*, 331, 778-782 (2011).
   DOI: 10.1126/science.1200729

4. Intermittent Pili-Mediated Forces Fluidize Neisseria meningitidis Aggregates Promoting Vascular Colonization. Bonazzi D, Lo Schiavo V, Machata S, Djafer-Cherif I, Nivoit P, Manriquez V, Tanimoto H, Husson J, Henry N, Chate H, Voituriez R, **Dumenil G**, *Cell* 174, 143-155 (2018).
   DOI: 10.1016/j.cell.2018.04.010

5. Colonization of dermal arterioles by Neisseria meningitidis provides a safe haven from neutrophils. Manriquez V, Nivoit P, Urbina T, Echenique-Rivera H, Melican K, Fernandez-Gerlinger MP, Flamant P, Schmitt T, Bruneval P, Obino D, **Dumenil G**, *Nat. Commun.* 12, 4147 (2021).
   DOI: 10.1038/s41467-021-24797-z