Structural Mass Spectrometry to study the interplay between proteasome complexes regulation mechanisms

I. Project's context, positioning and objectives

Context. Multiprotein complexes play various key roles in diverse biological processes. Among them, the 26S proteasome is a very well conserved multi-subunit protein assembly that presents structural and functional heterogeneities. Its main function, through the ubiquitin-proteasome system, is to **determine the turnover rate of intracellular proteins**, but specific other functions, such as the generation of functional peptides, were recently described[1-2] resulting from the association with different regulators (19S, PA28αβ, PA28γ, PA200). **Its dysregulation can be cytotoxic** and has been associated to **several neurodegenerative diseases and cancers**. We are aware of three main ways to tune its proteolytic activity: the interaction of the 20S catalytic core with dedicated regulators, the replacement of the standard (Std) catalytic subunits (β1, β2 and β5) by inducible immunological subunits that **form the immunoproteasome (i20S) upon inflammation** for instance, and finally by post-translational modifications (PTM). Three proteasome inhibitors targeting its chymotrypsin-like activity have been approved by the FDA since 2003 in the treatment of **multiple myeloma**[3-5]. However, a better understanding of proteasome regulation would allow **more specific therapies that target subtypes of the proteasome**, avoiding the unnecessary shutdown of downstream pathways that may cause deleterious side-effects[4-5]. Specific inhibition of the immunoproteasome was recently shown to reduce graft rejection[6] and suppress the progression of colorectal cancer in mice[7].

Objectives This project aims at **better understanding the complex interplay between the three distinct proteasome regulation pathways.** To do so, we will first characterize in-depth the proteasome through the definition of its proteoform repertoire, associated regulators and partners, in the context of **Inflammatory Bowel Diseases (IBD)**. Second, we will investigate the molecular basis explaining the preferential interaction between 20S subtypes and their different regulators. Finally we will screen new potential specific activators or inhibitors of the different 20S proteasome subtypes.

Strategies. The complexity of the proteasome complexes composition is a real challenge and **widely-used bottom-up proteomics approaches are not able to correlate this structural diversity with specific functions**. Although very powerful for protein identification and quantification, they do not account for PTM combination leading to various proteoforms[8]. To tackle this loss of information we will analyze these proteins entirely rather than after enzymatic digestion. We will employ state-of-the-art mass spectrometers to realize this **top-down approach** and have access to the many combinations of proteoforms present in a cellular extract and identify regulators that are specific to certain subtypes of the proteasome. We will **study this proteasome heterogeneity in the context IBD** as we know that the i20S will be recruited, at least partially, upon inflammation. We will use another innovative approach, **Hydrogen-Deuterium Exchange (HDX) MS,** to compare the **conformations of the Std vs. i20S proteasomes in the absence and presence of the PA28αβ and PA28γ regulators.** The same method will also be used to **identify binding interfaces of newly described Proteasome Interacting Proteins** (PIP)[9-10] and **study the binding of specific and non-specific inhibitors** to the Std vs. i20S. Finally, we will set out to identify new activators or inhibitors of the different proteasomes using a high-throughput workflow based on the chemical, fragment and peptide-based libraries available at the institute (PICT Platform). We are confident that these innovative structural MS methods[11-12] will shed a new light on our understanding of the proteasome regulation by studying **how its molecular and structural diversity can explain its different functions**. Furthermore, we will undoubtedly gather structural information that will be useful for the **design of new specific inhibitors.**

II. Methods

- Cell culture,
- Protein analysis (SDS-PAGE, Western Blot),
- Protein complexes purification (immunopurification) and biochemical enrichment (Size Exclusion Chromatography),
- Proteasomal enzymatic activity measurement (Fluorescence-based kinetic assay),

- Absolute proteasome assay using targeted mass spectrometry (Multiplexed Reaction Monitoring) and an isotope-dilution approach,
- Bottom-up and top-down proteomics (nanoHPLC ESI-MS/MS on different types of Orbitrap mass spectrometers),
- Mass spectrometry-based differential quantification and statistical analysis.
- Hydrogen-deuterium exchange coupled to Mass Spectrometry

III. Team

Team. **The Proteomics and Mass Spectrometry of Biomolecules team led by Odile Burlet-Schiltz has spent the last 15 years using proteomics to investigate the proteasome complexes**[9,10,13,14] that are a therapeutic target of choice because of their implications in many pathologies. A better description of the different forms of the proteasome will lead, in the long run, to **more specific treatments** generating less side effects. The **expertise of the scientific coordinator** will thus be supported by **a decade of experience in proteasome purification** and proteomics analysis in the team. Our team also counts with highly skilled and experienced engineers and bio-informaticians who will support the PhD student to be recruited, in terms of sample preparation, instrument handling and data analysis. The **team of F. Barreau and A. Ferrand** (INSERM U1220, Toulouse), who will provide us biological samples, is also renowned in the field of IBD and fully committed to this collaboration.

Co-supervisor. Julien Marcoux spent **more than 12 years using mass spectrometry** as a tool for the structural characterization of soluble and membrane proteins & protein complexes. He was recruited as a CNRS researcher in 2014 **in order to develop Structural MS methods** in the team. These methods are highly complementary to the classical proteomics workflow already well established by the team.

IV. References (articles from the team are in bold)

1 Liepe J *et al.* (2016) “A large fraction of HLA class I ligands are proteasome-generated spliced peptides” Science 354(6310):354-358

2 Dasgupta S *et al.* (2016) Analysis of the Yeast Peptidome and Comparison with the Human Peptidome” Plos One 11(9):e0163312

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4 Kuhn DJ *et al.* (2007) “Potent activity of carfilzomib, a novel, irreversible inhibitor of the ubiquitin-proteasome pathway, against preclinical models of multiple myeloma” Blood 110(9):3281-90.

5 Kupperman E *et al.* (2010) “Evaluation of the proteasome inhibitor MLN9708 in preclinical models of human cancer” Cancer Res 70(5):1970-80.

6 Li J *et al.* (2018) “Immunoproteasome inhibition prevents chronic antibody-mediated allograft rejection in renal transplantation” Kidney Int. 93(3):670-680.

7 Koerner J *et al.* (2018) “Inhibition and deficiency of the immunoproteasome subunit LMP7 suppress the development and progression of colorectal carcinoma in mice” Oncotarget. 8(31):50873-50888.

8 Smith LM *et al.* (2013) “Proteoform: a single term describing protein complexity” Nature 10(3):186-187.

9 Jonik-Nowak *et al.* (2018) “PIP30/FAM192A is a novel regulator of the nuclear proteasome activator PA28γ” PNAS 115(28):E6477-86.

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11 Marcoux J and Robinson CV (2013) “Twenty years of gas phase structural biology” Structure 21(9):1541-1550.

12 Marcoux J and Cianférani S (2015) “Towards integrative structural mass spectrometry: benefits from hybrid approaches” Methods 89:4-12.

13 Fabre B *et al.* (2014) “Label-Free Quantitative Proteomics Reveals the Dynamics of Proteasome Complexes Composition and Stoichiometry in a Wide Range of Human Cell Lines” J Proteome Res 13:3027–3037.

14 Fabre B *et al.* (2015) “Deciphering preferential interactions within supramolecular protein complexes: the proteasome case” Mol Syst Biol 11:771–771.