Study of the largest and most heterogeneous macromolecular complex by HDX-MS, bringing new important mechanistic insights in proteasome regulation

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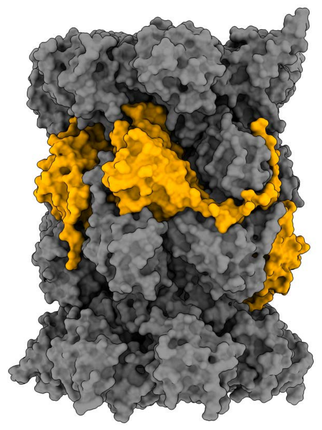
INTRODUCTION

Background

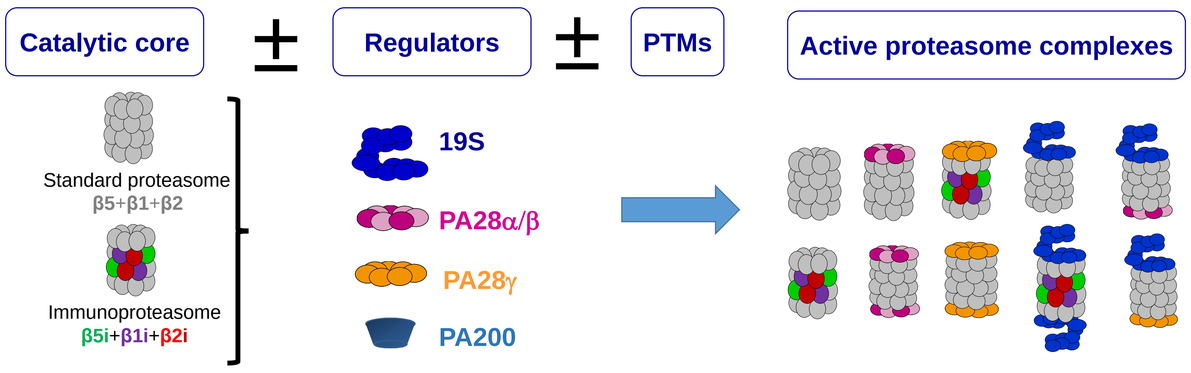
The 20S proteasome is composed of four heptameric-stacked rings. It degrades proteins in a tightly controlled fashion, thereby directly regulating intracellular concentration of cytokines and hub proteins, and may generate immunogenic peptides. Alteration of its activity can lead to cancers, heart and auto-inflammatory diseases. It can be regulated by replacing its constitutive catalytic subunits and/or by interacting with different activators. However, whether its catalytic subunits composition favors the interaction with a particular regulator is still unclear.

Methods and Results

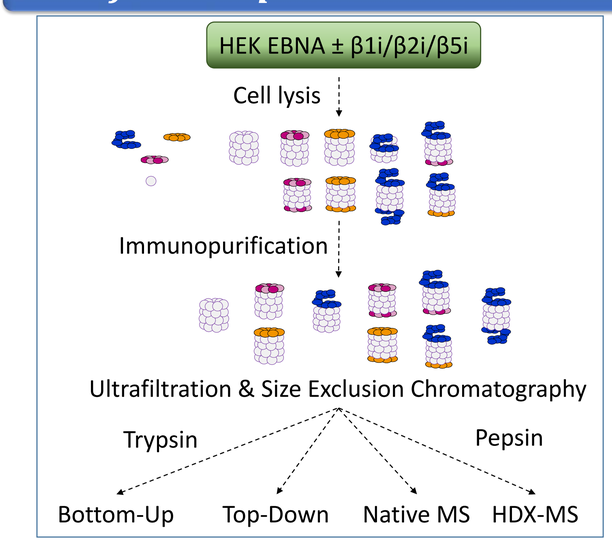
We utilized HDX-MS to investigate the impact of the catalytic subunit composition of the 20S on its structure and association to specific activators. Human standard and immuno proteasomes were deuterated alone or bound to the PA28αβ/PA28γ activators. We successfully optimized the classical HDX-MS workflow in terms of sample preparation, chromatography and MS acquisition to work on both poorly concentrated and very heterogeneous protein complexes. The average sequence coverage was excellent: 82% for twenty ~30kDa monomers. Our dataset suggests a reciprocal crosstalk between the inner and outer rings that not only represents a methodological breakthrough but also brings invaluable insights into the proteasome dynamics and regulation.



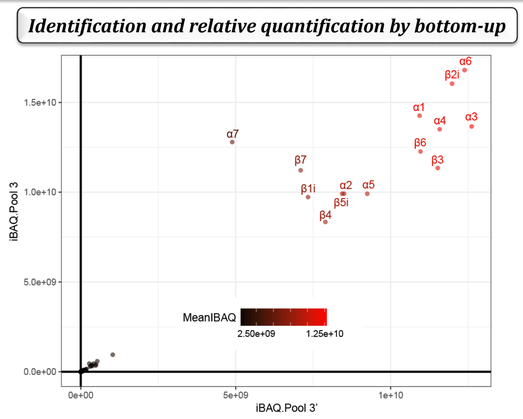
Proteasome complexes diversity



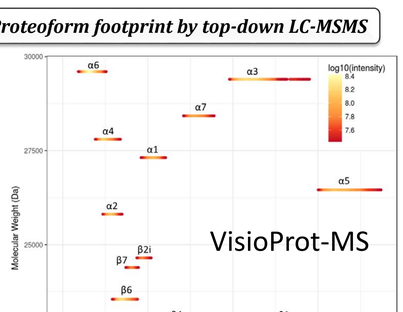
Purified complexes characterized by proteomics and structural MS



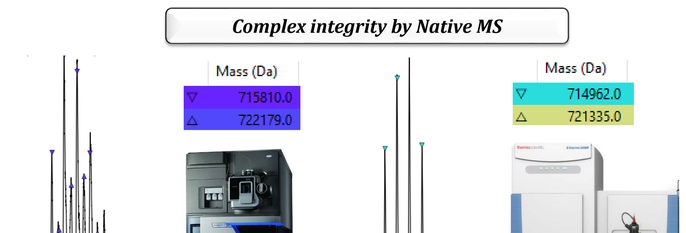
Identification and relative quantification by bottom-up



Proteoform footprint by top-down LC-MSMS



Complex integrity by Native MS



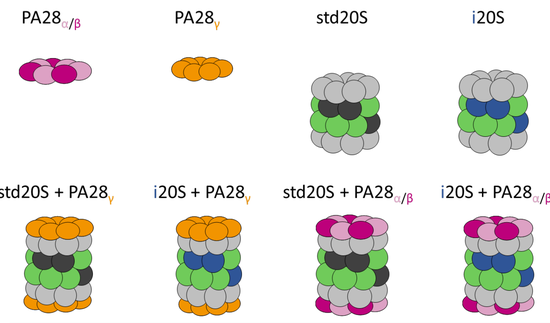
https://masstools.ipbs.fr/mstools/visioprot-ms/

Locard-Paulet M et al, (2019) BioInformatics

Hydrogen-Deuterium Exchange MS of the 20S ± PA28 activators

The sample preparation, chromatographic, MS acquisition, and data analysis steps had to be optimized for the analysis of such a large, heterogeneous and poorly concentrated sample. 4 pmol are required per timepoint (3.15 pmol injected).

Comparison of 6 conditions



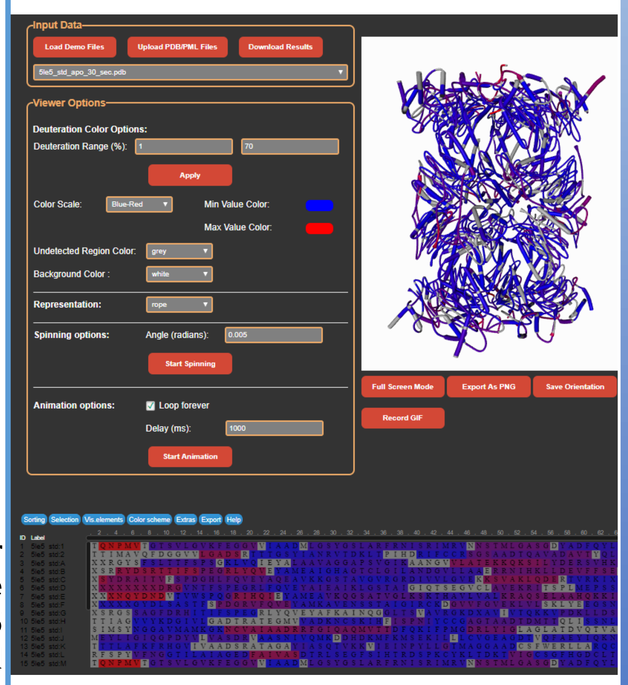
- 390 690 spectra acquired !
- 261 090 spectra validated manually !
- 767 peptides validated manually !

We obtained an average of 38 peptides per subunit corresponding to an average sequence coverage of 82% for each subunit in all the 6 tested conditions (better numbers on individual conditions)

Need to develop a new visualization tool

HDX-Viewer: open-access web application for the instant and interactive visualization of HDX-MS data

https://masstools.ipbs.fr/hdx-viewer



Bouyssie et al, (2019) BioInformatics *In Revision*

HDX-MS analysis of PA28γ vs. PA28αβ

* Strong protection of α-helices 1-4
* Proteasome facing interface and channel entrance strongly deuterated
* PA28γ and PA28αβ models display very flexible loops forming an antechamber (confirmed by preliminary Cryo-EM data on PA28γ) collab. P. Bron, CBS, Montpellier.

* Only difference observed in α-helix 4 (more dynamic in PA28α compared to PA28β and PA28γ)

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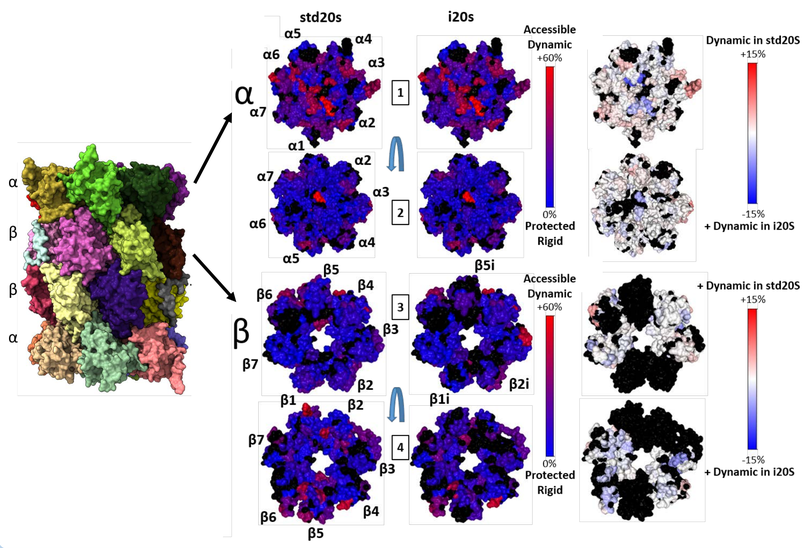
HDX-MS analysis of std20S vs. i20S shows subtle dynamics discrepancies



* Faster and higher deuteration of the solvent facing α-ring interface compared to any other ring interface.
* N-ter of α subunits (channel entrance) are very accessible/ dynamic
* Other "dynamic patches"
* N-ter of α1/2 subunits more accessible/ dynamic in i20S
* C-ter of α3 more dynamic in std20S than in i20S

→ Inner to outer ring allosteric effect

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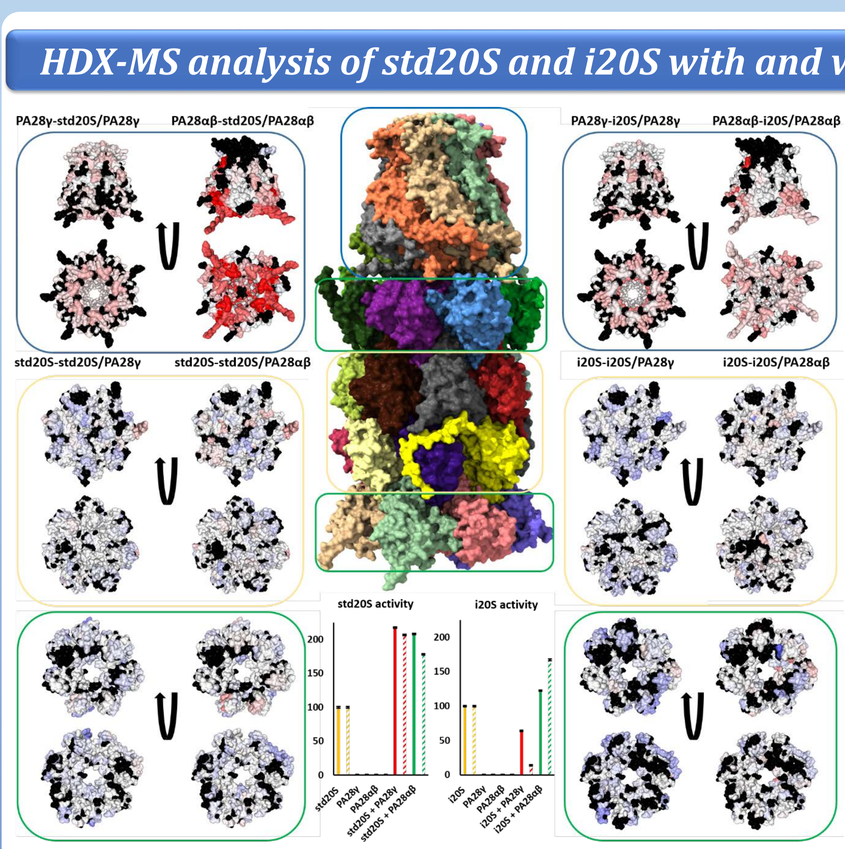
HDX-MS analysis of std20S and i20S with and without PA28 regulators



* Activation loop of PA28γ and PA28α protected by std20S but not i20S
* Activation loop of PA28β never protected
* Change in helix α1 kink observed for PA28γ and PA28α but not PA28β
* Most protected regions found at the 20S facing interface
* C-ter of α3 highly protected by PA28αβ and PA28γ in std20S but not in i20S
* Subunits β2/3/4, facing α3 are less dynamic. Their other side (β-β ring interface) is more dynamic.

→ Outer to inner ring allosteric effect

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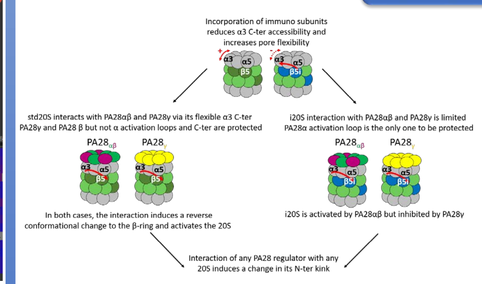
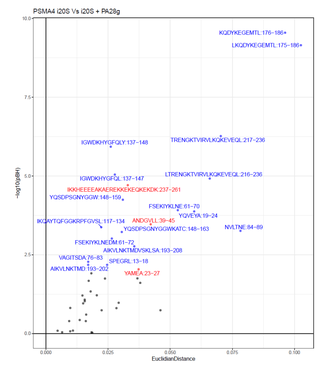
Conclusions and Perspectives



* Methodological breakthrough
* Wealth of structural information
* Finish Statistical analysis
* Ligand binding analysis by HDX-MS
* Proteasome Interacting Proteins (>300!) binding interfaces
* Other regulators (PA200, 19S)
* Other proteasome subtypes



Locard-Paulet M, Parra J, Albigot R, Mouton-Barbosa E, Bardi L, Burlet-Schiltz O, **Marcoux J (2019)** "VisioProt-MS : interactive 2D maps from intact protein mass spectrometry" **BioInformatics** 35(4):679-681

Bouyssié D*, Lesne J*, Locard-Paulet M, Albigot R, Burlet-Schiltz O, **Marcoux J (2019)** "HDX-Viewer: interactive 3D visualization of Hydrogen-Deuterium eXchange data" **BioInformatics** *In Revision*

Lesne J, Parra J, Locard-Paulet M, Chavent M, Bouyssie D, Zivković D, Menneteau T, Coux O, Bousquet-Dubouch MP, Burlet-Schiltz O, Bron P, **Marcoux J (2019)** "A reciprocal crosstalk between the inner and outer rings of the 20S : the missing gap in proteasome regulation" *In Preparation*