A diversity of proteasome complexes but not at random: a way to regulate proteasome function

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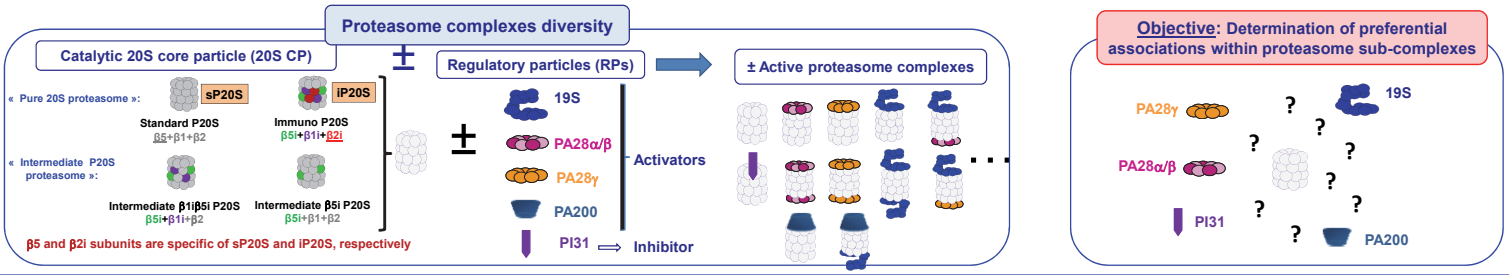
INTRODUCTION

One challenge of systemic biology is to characterize the complex nature of supramolecular protein machineries. Proteasome particles display high heterogeneity because they are formed by the dynamic association of several sub-complexes, a 20S core particle (20S CP), either single or associated to one or two regulatory particles (RPs) of identical or different protein composition. The 20S CP, which presents an α7β7β7α7 barrel-like structure, can itself be found in the eukaryotic cell as four different sub-types, the major forms being the standard proteasome (sP20S) and the immunoproteasome (iP20S) which differ in their catalytic beta subunits composition. In mammals, four different activators, the 19S, PA28αβ, PA28γ, and PA200 particles, are known to dramatically increase 20S proteasome activity through binding with the alpha ring while the PI31 protein is known to inhibit the 20S proteolytic complex. The 26S proteasome is a particular form where the 20S CP is capped by two 19S RPs, forming a 2.4 MDa complex.

Proteasome complexes therefore exhibit a high degree of heterogeneity in their overall subunit composition. Given the broad function of proteasomes, in quality control, antigenic peptide generation or short-lived protein-tuned regulation, the cell is likely to adapt proteasome plasticity and dynamics to meet specific subcellular needs or to respond to stress or other stimuli. Therefore, understanding the structure/function relationship controlling proteasome activity is of major interest in biology. Recent structural studies (1) clearly suggest that changes in active sites configurations, as found in sP20S and iP20S, might affect binding affinities for RPs. It would thus be of great interest to characterize the functional relevance of proteasome diversity through the identification of putative preferential associations within proteasome sub-complexes.

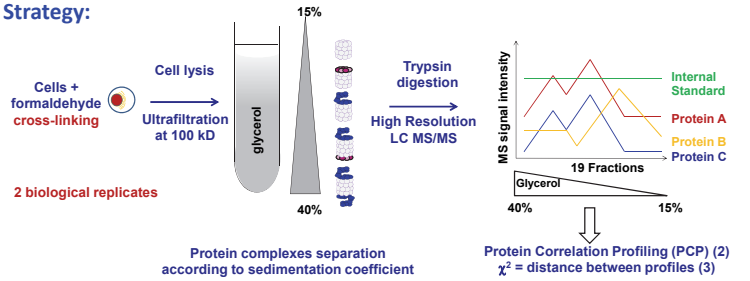
The aim of the present study was to decipher proteasome heterogeneity through modern label-free quantitative proteomics. Using **Protein Correlation Profiling and High Resolution Mass Spectrometry (PCP-HR-MS)** (2) of glycerol gradient-separated proteasome complexes, we could first reveal a previously unreported preferential association of immunoproteasome (iP20S) with the PA28αβ RP. Then through the development of a new workflow combining PCP-HR-MS and **AP-MS (Affinity Purification Mass Spectrometry)**, we could increase the sensitivity of detection of proteasome regulators and thus go deeper into proteasome characterization. Indeed, by correlating proteins abundances across a large set of 24 proteasome samples immunopurified from nine different human cell lines, we observed that the two main 20S proteasome subtypes, sP20S and iP20S, interact with a different subset of regulators. For instance, the PA200 and PI31 regulators were found highly correlated with the sP20S whereas the PA28αβ RP was matched to the iP20S. The 19S RP interacted equally with both 20S CP subtypes.

Some of these preferential interactions were validated by physiologically changing the proportions of both 20S CP subtypes in assembled proteasomes thanks to IFNγ stimulation. This novel integrated proteomic workflow provides a valuable tool to better understand the dynamic and complex nature of molecular systems.

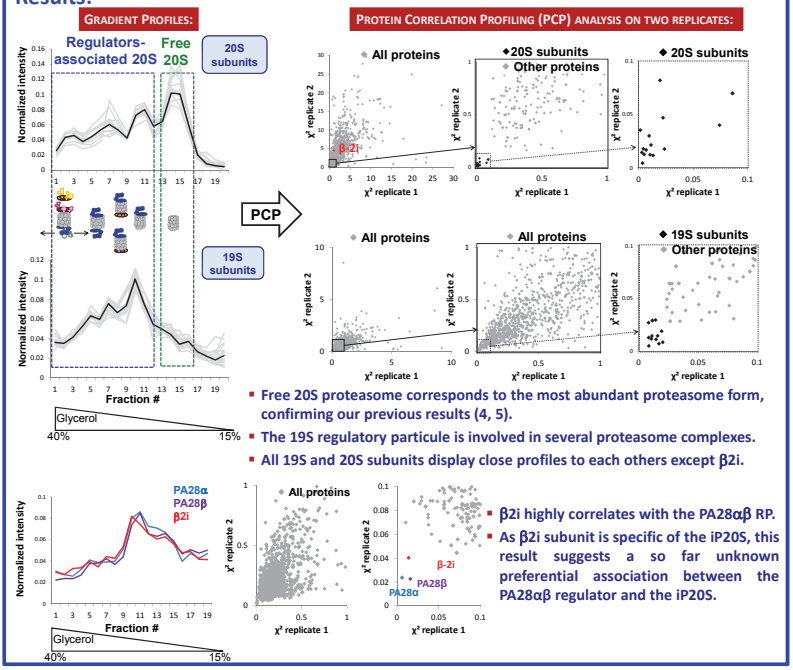


I- Protein Correlation Profiling High Resolution Mass Spectrometry (PCP-HR-MS) of glycerol gradient-separated proteasome complexes

Strategy:



Results:



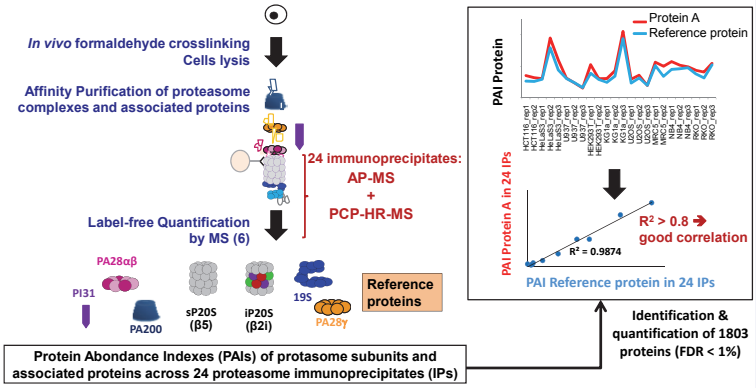
- Free 20S proteasome corresponds to the most abundant proteasome form, confirming our previous results (4, 5).
- The 19S regulatory particule is involved in several proteasome complexes.
- All 19S and 20S subunits display close profiles to each others except β2i.

- β2i highly correlates with the PA28αβ RP.
- As β2i subunit is specific of the iP20S, this result suggests a so far unknown preferential association between the PA28αβ regulator and the iP20S.

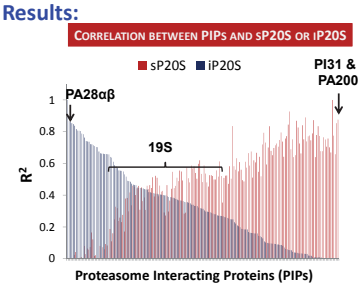
II- Novel combination PCP-HR-MS and AP-MS for deeper characterization of proteasome sub-complexes

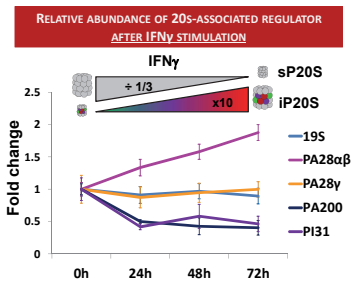
Strategy:

HCT116, HeLa S3, U937, HEK 293T, KG1a, U2OS, MRC5, NB4 and RKO human cell lines
2 or 3 biological replicates per cell line → 24 immunoprecipitates



Results:





- Out of 1803 proteins identified, 193 correlate with at least one reference (R^2 value > 0.8) and, of which, 120 are known Proteasome Interacting Proteins (PIPs)
- Standard proteasome (sP20S) and immunoproteasome (iP20S) interact with a different subset of regulators: sP20S with PA200 and PI31 - iP20S with PA28αβ.
- The 19S RP does not have any preference to any of the two sP20S and iP20S subtypes and correlates with the non catalytic subunits of the 20S proteasome (data not shown).
- When the relative proportion of sP20S and iP20S was physiologically changed using IFNγ stimulation, no significant variations of 20S-associated 19S and PA28γ were noticed while the trends of PA28αβ on one hand and of PA200 and PI31 on the other hand followed the ones of the iP20S and of the sP20S, respectively.

CONCLUSION

Here, using a new developed method based on the combination of affinity purification (AP) and protein correlation profiling (PCP) associated to high resolution mass spectrometry (HR-MS), we comprehensively characterized proteasome heterogeneity and identified preferential associations within proteasome sub-complexes. In particular, we showed for the first time that the two main proteasome sub-types, standard proteasome (sP20S) and immunoproteasome (iP20S) , interact with a different subset of important regulators. This trend was observed in very diverse human cell types and was confirmed by changing the relative proportions of both 20S proteasome forms using interferon-γ.

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