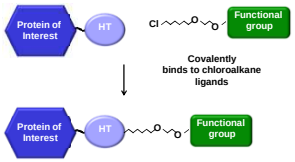
Integrating commercial products and services to rapidly isolate and characterize the human proteasome

Brad Hook[1], Jacqui Méndez[1], Richard Jones[2], Michael Ford[2], Danette Daniels[1], Marjeta Urh[1], and Trista Schagat[1].
[1]Promega Corporation 2800 Woods Hollow Road, Madison, WI U.S.A. 53711
[2]MS Bioworks, LLC 3950 Varsity Drive Ann Arbor, MI 48108

1. Abstract and introduction

The understanding of protein complex assembly and mapping of protein interactions has rapidly grown in recent years due to significant advances in mass spectrometry. Here, we sought to identify subunits of the human proteasome using an assay design that minimized time by using integrated commercial products and services. Critical steps in the project used products and services from Kazusa DNA Research Institute, Promega Corporation, and MS Bioworks. The initial construct was purchased from Kazusa institute which allowed for rapid initiation of the project with no need for cloning. This construct contained a fused proteasome subunit to HaloTag®, a novel protein fusion tag which allows for highly specific, oriented, and covalent immobilization of proteins on surfaces. After transfection using Fugene® HD into HEK293 cells, the proteasome complex was isolated using the HaloTag® Mammalian Pulldown system and analyzed by using LC-MS/MS. The proteasome was assayed for function using a luciferase based assay from Promega Corporation. Protein: protein interactions were mapped using cell-free protein expression. Using these commercially available products and services allowed for rapid analysis of this multi-protein complex.

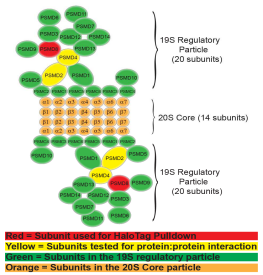
2. HaloTag technology



- HaloTag (HT) is a genetically encoded 33kDa protein fusion tag.
- Engineered to covalently bind various ligands, imparting multi-functionality.

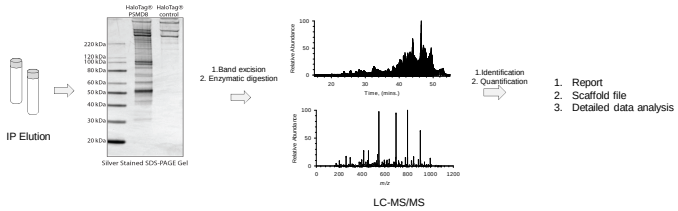
- HaloTag applications include:
  - HaloTag surface ligands
    - Protein display
    - Protein purification
    - Interaction analysis
  - HaloTag fluorescent ligands
    - Cellular imaging
    - Gel analysis
    - Quantitation
  - HaloTag reactive ligands
    - Attach modifications
    - Attach to particles
    - Attach to surfaces

3. Human Proteasome Model



- Chen *et al*. "Subunit-subunit interactions in the 26S Proteasome" Proteomics 2008, 8, 508-520
- Guerrero *et al*. "Characterization of the Proteasome Interaction Network Using a QTAX-based Tag-team Strategy and Protein Interaction Network Analysis" PNAS 2008, 36, 13333-13338

4. Protein Complex Analysis Workflow



- 20µL of the IP elution (1-2µg total protein) was separated on a 10% Bis-Tris NuPage gel (Invitrogen) in the MES buffer system.
- The 2cm gel migration window was excised into ten equally sized segments.
- In gel digestion was performed using a robot (ProGest, DigiLab) and Promega sequencing grade trypsin.
- Each digest is processed by LC-MS/MS using 30 minute reverse phase gradient.

5. Identification of Proteasome Subunits

sub-unit	PSMD8 HT-TEV	Control HT-TEV	sub-unit	PSMD8 HT-TEV	Control HT-TEV	sub-unit	PSMD8 HT-TEV	Control HT-TEV	sub-unit	PSMD8 HT-TEV	Control HT-TEV
PSMA1	0.0034	0	PSMB2	0.0046	0	PSMC1	0.0096	0	PSMD1	0.0104	0
PSMA2	0.0039	0	PSMB3	0.0044	0	PSMC2	0.0091	0	PSMD2	0.0105	0
PSMA3	0.0039	0	PSMB4	0.0025	0	PSMC3	0.0113	0	PSMD3	0.0114	0
PSMA4	0.0023	0	PSMB5	0.0042	0	PSMC4	0.0096	0	PSMD4	0.0065	0
PSMA5	0.0035	0	PSMB6	0.0021	0	PSMC5	0.0109	0	PSMD6	0.0127	0
PSMA6	0.0048	0	PSMB7	0.0020	0	PSMC6	0.0090	0	PSMD7	0.0085	0
						PSMD9	0.0000	0			
						PSMD10	0.0084	0			
						PSMD11	0.0111	0			
						PSMD12	0.0084	0			
						PSMD13	0.0108	0			
						PSMD13 similar	0.0105	0			
						PSMD14	0.0058	0			

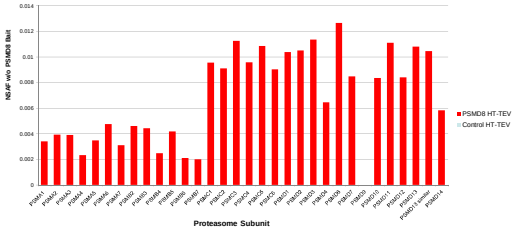
Normalized Spectral Abundance Factor (NSAF)

NSAF = $(SpC/Mw)/\Sigma(SpC/Mw)_n$

SpC = Spectral Counts
Mw. = Protein molecular weight in kDa
N = Total number of proteins

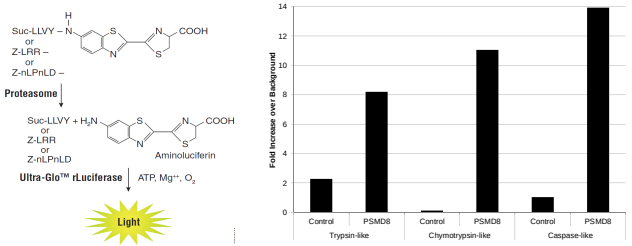
- Proteasome subunits were isolated using the HaloTag Mammalian Pull-Down System
- 33 of 34 proteosome subunits were identified by LC-MS/MS. PSMD9 was not detected.

6. Visualization of Subunits Abundance



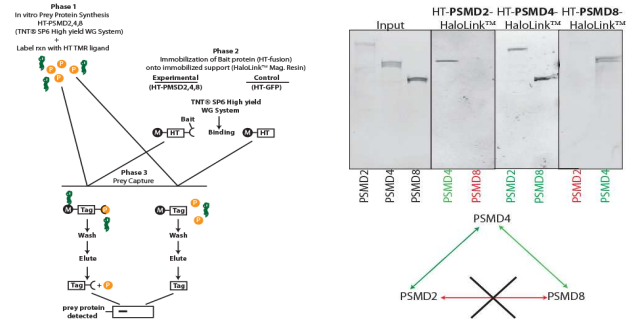
- Proteasome subunits were isolated using the HaloTag Mammalian Pull-Down System
- 33 of 34 proteosome subunits were identified by LC-MS/MS. PSMD9 was not detected.
- The Normalized Spectral Abundance Factor (NSAF) approach enables direct comparison of protein abundances within and across sample sets

7. Proteasome Glo™ Assay



- Proteasome-Glo™ assay provides a sensitive and easy assay for analysis of isolated complexes
- The HaloTag PSMD8 pull-down eluates exhibit all three proteasome-like activities; whereas the control pull-down had little proteasome-like activity

8. Cell-Free Expression



- Cell-Free protein expression allowed for direct protein:protein interaction mapping without the need to reclone or purify any proteins. (HT-GFP control not shown.)
- The HaloTag provided a sensitive and fast way to detect the proteasome subunits
- Direct interactions between PSMD2 and PSMD4 and also between PSMD4 and PSMD8 were found

9. Summary

- Using products from Promega Corporation and services from the Kazusa Institute and MS Bioworks, we quickly isolated human proteasome, tested activity and characterized subunit interactions.
- HaloTag technologies allowed rapid isolation of a functional proteasome from HEK293, immobilization for protein:protein interaction studies, and fluorescent labeling for detection after SDS-PAGE
- Cell-Free protein expression is an easy and fast method for confirming and mapping protein:protein interactions discovered by mass spectrometry

If you have any questions or comments please contact info@msbioworks.com