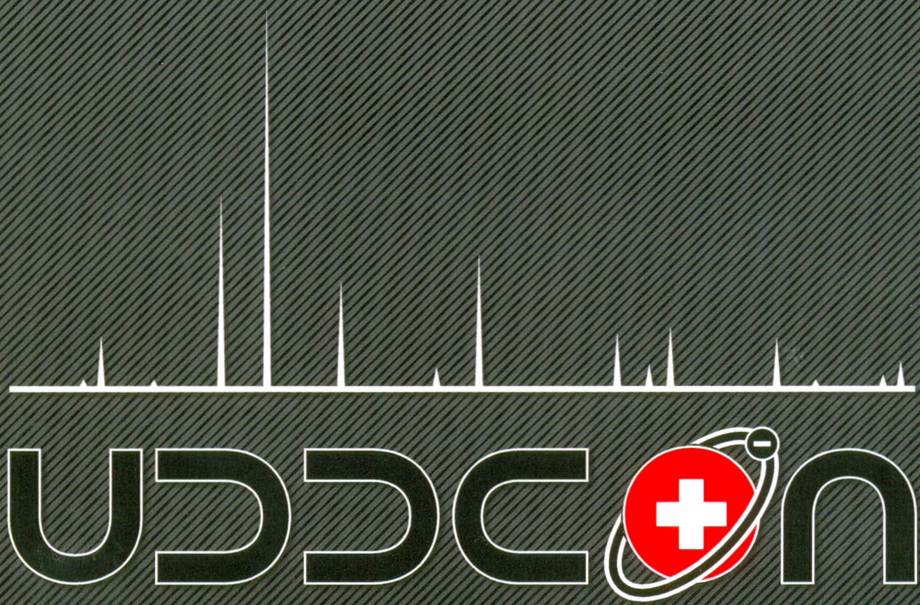

8th Uppsala Conference (UppCon) & School on Electron Capture and Transfer Dissociation

February 6-10, 2011 • Villars-sur-Ollon • Switzerland

2003 Sweden-Finland | 2004 Scotland | 2005 Washington | 2006 Hong Kong | 2007 France | 2008 Wisconsin | 2009 Japan | 2011 Switzerland

GENERAL INFORMATION

UppCon History

Year	Organizer	Location
2012	To be annnounced…	To be annnounced…
2011	Yury Tsybin	Villars-sur-Ollon, Switzerland
2009	Takashi Baba	Nara, Japan
2008	Joshua Coon	Madison, WI, USA
2007	Guillaume van der Rest	Paris, France
2006	Dominic Chan	Hong Kong, Hong Kong
2005	David Goodlett	Seattle, WA, USA
2004	Pat Langridge-Smith	North Berwick, Scotland
2003	Roman Zubarev	Sweden-Finland, on a ferry

Venue. The 2011 conference will take place in the **Eurotel Victoria** hotel located in the picturesque village Villars-sur-Ollon in the Swiss Alps. All lectures will be held in Villars I & II halls and poster/exhibition session in the adjacent Les Diablerets Hall (Eurotel Victoria, ground floor). Posters and exhibitor tables will be open for viewing from Monday, February 7th for the entire meeting. Breakfast/Lunch/Dinner will be served in diverse restaurants of Eurotel Victoria. All mobile phones must be turned off or set to vibrate during all oral sessions. Courtesy is expected.

Posters must be set up beginning on Sunday, February 6th or Monday, February 7th. All posters need to be in place by Monday, February 7th at 20.30. Posters must be removed no later than Thursday, February 10th at 12.00. Please consult the Poster Program for the poster numbers.

Gala Dinner will take place in restaurant Botta at the altitude of 3'000 m, on the top of a Glacier on Wednesday, February 9th. Gala Dinner costs are included into the registration fee. There are 2 ways to get to the **Glacier 3'000** (www.glacier3000.ch). First, you can ski from Villars-sur-Ollon all the way to Les Diablerets and then take a short bus ride to the Glacier 3'000. Second, you can take a bus ride from outside Hotel Eurotel Victoria (conference hotel) at 13.30. We will all return by the same bus on Wednesday night to the conference hotel. You can keep your belongings in the bus.

Snow activites. The region of Villars-sur-Ollon offers a multitude of snow activities (if there is snow…). You can rent the required equipment at the shop « Sport's House» (www.sportshouse.ch) just outside the conference hotel. **Mention «UppCon» to get 20% discount!**

The conference secretariat can be found in the lobby of Eurotel Victoria during the entire meeting or by calling the mobile phone number to reach us in case of an urgent question.

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STRUCTURE OF PROTEASOMAL CORE PARTICLE OF *H. volcanii* BY CHEMICAL CROSS-LINKING AND MASS SPECTROMETRY

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Between a few MS techniques used for structural analysis of proteins, chemical cross-linking coupled with mass spectrometry (CXMS) emerged as a method which can yield site-specific low resolution structure information on the distance constraints with sample quantities that are 2-3 order of magnitude less than required for X-ray and NMR and obtained in less time. The general principle of CXMS is the covalent capture of juxtaposed amino acids using a variety of cross-linking reagents. CXMS can provide intra- and intermolecular distance constraints that can be used (respectively) to resolve protein folding in a monomer protein /subunit, and interactions at molecular interfaces in protein complexes. A new CXMS analytical approach based on high performance mass spectrometry to generate tandem mass spectra and the open modification search strategy to interpret the data has been recently reported (Singh, 2008, Sanowar, 2010). Distance determinations obtained by CXMS technique lead to important advances in mapping the protein topography in low resolution structures refined by computational methods. *Ab initio* and comparative modeling of protein complexes result in a number of possible structure, thus distance constraints generated by CXMS could facilitate the evaluation and/or construction of modeled structure.

Haloferax volcanii is a haloarchaeon which encodes at least three protein components associated with the 20 S protelytic core particle of proteasome system. The 20S proteolytic core particles (CPs) of proteasomes from *H. volcanii* catalyze the ATP-independent proteolysis of unfolded proteins. The CP is a cylindrical protein complex of 4 heptameric rings organized in $\alpha7\beta7\beta7\alpha7$ stoichiometry. The α-type subunits form the outer rings are presumed to limit the access of protein substrates into and out of the central proteolytic chamber formed by the two inner rings of β-type subunits. The archaeon *H.volcanii* synthesizes two different α-subunits, $\alpha1$ and $\alpha2$, having the potential to make 3 different CPs: 2 symmetric ($\alpha1\beta\beta\alpha1$, $\alpha2\beta\beta\alpha2$) and one asymmetric ($\alpha1\beta\beta\alpha2$) (Kaczowka, 2003).

Using high performance mass spectrometry to generate tandem mass spectra and the open modification search strategy to interpret the data, chemically cross-linked proteins in the symmetric $\alpha1\beta\beta\alpha1$ and $\alpha2\beta\beta\alpha2$ core particle of *H. volcanii* proteasomes were analyzed. Two commercial chemical cross-linkers: zero length EDC and homobifunctional amine-specific BS2G were investigated to determine the juxtaposed amino acids in the CP and to validate three-dimensional protein models generated by comparative modeling. CXMS of $\alpha1\beta\beta\alpha1$ by BS2G showed one interpeptide and one intrapeptide cross-links, whereas in $\alpha2\beta\beta\alpha2$ no any interpeptide was found. Using EDC three interpeptide cross-links were found in $\alpha1\beta\beta\alpha1$ and two in $\alpha2\beta\beta\alpha2$, suggesting significant difference in CP's. Distance constraints obtained by CXMS were validated using a new software platform called MSX-3D (Heymann, 2008).

[1] Singh P, *et. al.*, Characterization of protein cross-links via mass spectrometry and an open-modification search strategy. *Anal. Chem. (2008), 80, 8799-8806.*
[2] Sanowar S, *et. al.*, Interactions of the Transmembrane Polymeric Rings of the Salmonella enterica Serovar Typhimurium Type III Secretion System. *MBio. (2010),1(3). pii: e00158-10.*
[3] Kaczowka S.J., *et. al.*, Subunit topology of two 20S proteasomes from *Haloferax volcanii*. *J. Bacteriol. (2003), 185, 165-174.*
[4] Heymann M, *et. al.*, MSX-3D: a tool to validate 3D protein models using mass spectrometry. *Bioinformatics (2008), 24, 2782-2783.*

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STRUCTURE OF PROTEASOMAL CORE PARTICLE OF *H. volcanii* BY CHEMICAL CROSS-LINKING AND MASS SPECTROMETRY

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Introduction

Between a few MS techniques used for structural analysis of proteins, chemical cross-linking coupled with mass spectrometry (CXMS) has emerged as a rapid method which can yield site-specific low resolution structure information on the distance constraints with sample quantities that are 2-3 order of magnitude less than required for X-ray and NMR. The general principle of CXMS is the covalent capture of juxtaposed amino acids using a variety of cross-linking reagents. CXMS can provide intra- and intermolecular distance constraints which can be used (respectively) to resolve protein folding in a monomer protein/subunit and interactions at molecular interfaces in protein complexes. A new CXMS analytical approach based on high performance mass spectrometry to generate tandem mass spectra and the open modification search strategy to interpret the data has been recently reported and validated (Singh, 2008, Sanowar, 2010). Distance determinations obtained by CXMS technique lead to important advances in mapping the protein topography in low resolution structures refined by computational methods. *Ab initio* and comparative modeling of protein complexes result in a number of possible structures, thus distance constraints generated by CXMS could facilitate the evaluation and/or construction of modeled structure.

Haloferax volcanii is a haloarchaeon which encodes at least three protein components associated with the 20 S protelytic core particle of the proteasome system. The 20S proteolytic core particles (CPs) of proteasomes from *H. volcanii* catalyze the ATP-independent proteolysis of unfolded proteins. The CP is a cylindrical protein complex of 4 heptameric rings organized in α7β7β7α7 stoichiometry. The α-type subunits that form the outer rings limit the access of protein substrates into and out of the central proteolytic chamber formed by the two inner rings of β-type subunits. The archaeon *H volcanii* synthesizes two different α-subunits, α1 and α2, having the potential to make 3 different CPs: 2 symmetric (α1ββα1, α2ββα2) and one asymmetric (α1ββα2) (Kaczowka *et al.*, 2003).

Using high performance mass spectrometry to generate tandem mass spectra and the open modification search strategy to interpret the data, chemically cross-linked proteins in the symmetric α1ββα1 and α2ββα2 core particle of *H. volcanii* proteasomes were analyzed. Two commercial chemical cross-linkers: zero length EDC and homobifunctional amine-specific BS2G were investigated to determine the juxtaposed amino acids in the CP and to validate three-dimensional protein models generated by comparative modeling. CXMS of α1ββα1 by BS2G showed one interpeptide and one intrapeptide cross-links, whereas in α2ββα2 no interpeptide cross-links were found. Using EDC three interpeptide cross-links were found in α1ββα1 and two in α2ββα2, suggesting significant differences in these CPs. Distance constraints obtained by CXMS were validated using a new software platform called MSX-3D (Heymann, 2008).

Material and Methods

Proteasome Purification

Proteasomes were purified by tandem Ni^{2+}-Sepharose (HiTrap chelating; Amersham Biosciences) and Streptactin (Qiagen) chromatography as previously described (3).

Cross-Linking Reactions

Proteasomes were cross-linked with: (i) bis[sulfosuccinimidyl] glutarate-d_0 (BS2G), (ii) 1-ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride (EDC). Cross-linking reagents were purchased from Pierce, ThermoScientific, USA.

Mass Spectrometry and HPLC

Peptide digests were analyzed by electrospray ionization in the positive mode on a hybrid linear ion trap-Orbitrap instrument (LTQ-Orbitrap, Thermo Fisher, San Jose, CA, USA). Peptides were separated by nanoflow HPLC (NanoAcquity, Waters Co., Milford., MA, USA). Homemade precolumns (100 μm i.d. x 25 mm long) packed with 200 A C_{18} stationary phase (5 μm C18AQ,Michrom) were used for peptide trapping. Analytical columns (75 μm x 210 mm long) packed with 100 A C_{18} stationary phase (5 μm, C18AQ, Michrom) were coupled to the mass spectrometer. Peptide mixture (0.5 μg) was loaded onto the precolumn at 4 μl·min^{-1} in 5% (v/v) acetonitrile with 0.1% (v/v) formic acid. Peptides were eluted by a linear gradient of A, water, 0.1 % formic acid and B, acetonitril, 0.1% formic acid, as follows: 0 min, A (95%), B (5%); 55 min, A (65%), B (35%); 60 min, A (15%), B (85%); 65 min, A (5%), B (95%); 75-90 min, A (95%), B (5%) . All MS survey scans were performed from m/z 400-2000, at a resolution of 60,000 (m/z) and ion population of 5×10^5. For tandem MS resolution was set to 7500, ion population to 2×10^4 and precursor isolation width to 4 m/z units. Data dependant analysis was performed by selection of the five most abundant precursors, rejecting singly, doubly, triple charged ions. Data redundancy was minimized by dynamic exclusion of previously selected precursor ions (-0.1/1.1 Da) for 45 s before being selected again for fragmentation.

MS Data Processing

Tandem mass spectral data were converted to .dta files and deconvoluted to the 2+ charge state precursor and 1+ charge state fragments by an in-house written Perl script (http://goodlett.proteomics.washington.edu). For CXL search, a database was formed using xComb v1.1 parameters : sequence in UniProt FASTA format; cleavages by trypsin with up to 2 missed cleavages; intra- and inter-protein crosslinks . Deconvoluted spectra were searched by Phenyx (GeneBio SA, Geneva, Switzerland) to identify cross linked peptides. Search parameters for Phenyx were as follows: databases created by xComb were added; taxonomy-root; scoring model-ESI-LTQ-Orbitrap (CID_LTQ_scan_ Orbitrap_6 ppm); parent charge- 1,2,3,4; modifications including methionine in oxidized and reduced forms and cysteine aklylated by iodacetamide; enzyme- do not cleave; missed cleavages-0; parent tolerance- 10 ppm; peptide thresholds: length ≥ 6, score ≥ 4.0, p-value ≤ 1.0 E-5; AC score of 4; turbo scoring: tolerance of 10.0 ppm, coverage ≥ 0.1, series of b,b++,y,y++ were used. File in .mgf format was submitted. The MS/MS fragmentation of cross linked peptides obtained from Phenyx search was analyzed to assign ion peaks, using MS2Assign, with threshold of 50 ppm.

Protein structures validation

To validate 3D protein models using mass spectrometry data a new tool MSX-3D, version 3.4.23 (http://proteomics-pbil.ibcp.fr/cgi-bin/msXsetup.pl), (Heymann, 2008) was used. Pairwise comparison of protein structures was established using DaliLite software (http://www.ebi.ac.uk/Tools/dalilite/index.html), (Holm, 2008). The coordinates of heptameric complexes of α1, α2 and β were obtained by DaliLite software using PDB entries 1j2p for α1, α2, and 1ryp and 1fnt for β. Molecular modeling package Vega ZZ was used to add hydrogens on ring structures and to calculate charge and potential using Charm for force filed and Gasteiger for charge (Pedretti, 2002). Energy minimized using AMMP force field implemented in VEGA ZZ

Conclusion

A recently reported new CXMS analytical approach was used to explore a model protein complex/ nanomachine isolated from the halophilic archaeon H. volcanii (Singh, 2008, Sanowar, 2010). Using high performance mass spectrometry to generate tandem mass spectra and the open modification search strategy to interpret the data, chemically cross-linked proteins in the symmetric α1ββα1 and α2ββα2 core particle (CP) of H. volcanii proteasomes were analyzed. Two commercial chemical cross-linkers: zero length EDC and homobifunctional amine-specific BS2G were investigated to determine the juxtaposed amino acids in the CP and to validate three-dimensional protein models generated by homology comparative modeling. Since the α1 and α 2 proteins share only 55.5% identity, a significant structural differences in the homoheptameric rings formed by these two proteins was predicted (Kaczowka, 2003). Indeed, significantly different CXMS profiles were observed for α1ββα1 and α2ββα2 CP's: β rings reminded unchanged in both symmetric CP preparation, giving the same cross-linked peptides, whereas cross-linked peptides from α1 and α2 differed suggesting a distinction in its topology. By MSX-3D software platform intramolecular distances in ab intio theoretical structure models from ModBase were validated for α1, β and α2 proteins, whereas intermolecular distances between cross-linked sites of proteins from complexes organized in (α1)7, β7, and (α2)7 rings of proteasomal core particle of H.volcanii were analyzed by models based on PDB entries 1j2p, 1fnt and 1j2p, respectively. Indeed, a significant difference in homology modeled 3D structures of proteasomal α1 and α2 hepatmeric ring structures were found, whereas inner β heptameric rings structures reminded unchanged in both α1ββα1 and α2ββα2 core particles of H. volcanii proteasomes. This study clearly reveals that two symmetric 20S proteasomes differ in topology. Finally, this study showed that even a small number of distance constraints obtained by CX-MS can assist in the determination of complex protein structures and facilitate recognition of on accurate model.

Acknowledgment

This work was funded in part by: NIH R01 GM057498 and DOE DE-FG02-05ER15650 to JMF. NIH R33CA099139-01, 1S10RR023044-01 and 1U54AI57141-01 to DRG. Fulbright Association and III 43004 to IMK.

Results and Discussion

Cross-linked peptides identified from 20S of symmetric α1ββα1 and α2ββα2 proteasomes after treatment with homobifunctional lysine reactive BS2G and zero length cross-linker EDC are shown in Table 1.

Table 1. Cross-linked peptides identified in symmetric α1ββα1 and α2ββα2 proteasome after treatment with BS2G and EDC

[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
				α1ββα1		
2945.56	5	7.6	BS2G	α1, 44-57 α1, 58-71	TPEGVVLAADKR- SPLMEPTSVEKIH	K54-K68
2453.28	4	5.7	EDC	α1, 44-57 α1, 58-71	TPEGVVLAADKR- SPLMEPTSVEK	K54-E67
3650.74	4	1.7	EDC	α1,150-171 α1, 58-68	LYETDPSGTPYEWKAVSIGADR – SPLMEPTSVEK	K163-E62/E67
2069.98	4	4.9	EDC	β, 69-82 β, 209-213	ASMGYMVSSKDVQK-SAVER	K78-E-212
				α2ββα2		
4164.12	4	2.4	EDC	α2, 116-148 α2, 88-94	TITDNIQESTQSGGTRPYGASLLIG GVENGSGR- KLVDFAR	K88- D119
2069.98	4	4.9	EDC	β, 69-82 β, 209-213	ASMGYMVSSKDVQK–SAVER	K78-E-212

Interpeptide cross-linking found in α1 subunit between K54-K68 is lacking in α2 subunit. When α1 and α2 were aligned it was found that K54 from α1 was exchanged with R53 in α2, so K-67 had not counterpart to form crosslink.

Figure 1. Alignment of α1 and α2 sequences using ClustalW. Accession numbers: α1 Q9V2V6 and α2 Q9V2V5. Conserved region are gray shaded.

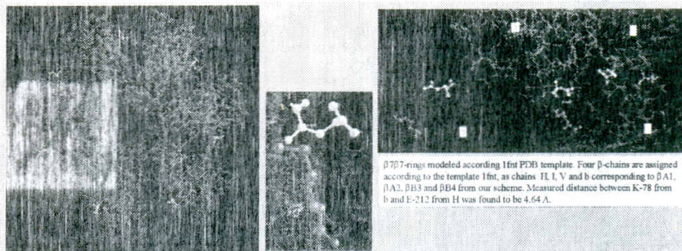
A new software platform MSX-3D for validation of a theoretical models based on CXMS data (Heymann, 2008) was used in order to validate interresidue distances obtained by open modification CXMS strategy. Observed cross-links were compared with predicted from homology modeled structure of subunits α1, β and α2.

Figure 2. MSX-3D prediction. Peptides TPEGVVLAADKRSR cross-linked with SPLMEPTSVEKIHK are on the distance of 9.45 A in a model structure of α1 subunit, based on PDB template 1j2p.

Table 2. Intramolecular cross-links and distances between cross-linked sites of α1, β and α2

[illegible]	[illegible]	Sub-unit	Cross-linked sites	Measured distance, A
BS2G	TPEGVVLAADKRSR- SPLMEPTSVEKIHK	α1	K54/K68	9.4
BS2G	ADELGDKLTKTGTTTVGIKTEEGVVLATDMRA SMGYMVSSK	β	K43 K47	6.1
EDC	TPEGVVLAADKRSR- SPLMEPTSVEKIHK	α1	K54/E67	6.9
EDC	LHKLDDALGAATAGHVADARK	α2	K70 D72, D73	6.6, 3.2

The coordinates of heptameric complexes of α1, α2 and β were obtained by DaliLite software using PDB entries 1j2p for α1, α2, and 1ryp and 1fnt for β. Coordinates of the PDB structures which had a high Z score and strong match with αa, βand α2 subunits were used to assemble protein complexes organized in ring structures: (α1)7, β7, and (α2)7 and doubled ring β7β7 by Vega ZZ molecular modeling software.



Heptameric α1-ring modeled according 1j2p PDB template, homology 51%. Measured distances between NZ of K163 (pink) and OE1 from E-62 (yellow) from two subunits were found to be 4.7 A.

β7β7-rings modeled according 1fnt PDB template. Four β-chains are assigned according to the template 1fnt, as chains H, I, V and b corresponding to βA1, βA2, βB3 and βB4 from our scheme. Measured distance between K-78 from b and E-212 from H was found to be 4.64 A.

Table 3. Intermolecular cross-links and distances between cross-linked sites of proteins from complexes organized in (α1)7, β7, β7β7 and (α2)7 rings from proteasomal core particle of *H.volcanii*

[illegible]	[illegible]	[illegible]	[illegible]	Measured distance, A
[illegible]	[illegible]SPLMEPTSVEK	α1-α1	K163/E61	4.8 (1j2p)*
[illegible]	[illegible]	β-β	K78 E212	4.6 (1fnt)
[illegible]	[illegible]GASLLIGGVENGSGR	α2-α2	K88 D119	2.3 (1j2p)

Li[illegible]

[1] [illegible] identification of protein cross-links via mass spectrometry and an open-modification search strategy. *Anal. Chem. (2008), 80,* [illegible]

[2] [illegible] Interactions of the Transmembrane Polymeric Rings of the *Salmonella enterica* Serovar Typhimurium Type III Secretion Sy[illegible]

[3] [illegible] Subunit topology of two 20S proteasomes from *Haloferax volcanii*. *J. Bacteriol. (2003), 185, 165-174.*

[4] [illegible] MSX-3D: a tool to validate 3D protein models using mass spectrometry. *Bioinformatics (2008), 24, 2782-2783.*

[5] [illegible] 2002. Vega: a versatile program to convert, handle and visualize molecular structure on Windows-based PCs. *J.* [illegible] 21 47-9.