

# Partial-Body Radiation Diagnostic Biomarkers and Medical Management of Radiation Injury Workshop

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The NYU Department of Pharmacology Mass Spectrometry Laboratory is a structural biology / nanochemistry laboratory devoted to the identification and study of tissue and body fluid biomarkers of vascular disease, tumors and putative metabolic pathways of apoptosis triggered by ionizing radiation, reactive oxygen species, ischemia and other insults. The laboratory instrumentation includes, MALDI TOF TOF, LCMS, AFM, and access to 12T FTMS, 900 MHz NMR, and 300 KEV TEM.

In the past year direct mass spectrometry identification of proteins and biomarkers of colorectal carcinoma, ischemic / stroke brain, environmental toxins, and competent in-vitro human embryos were reported by the laboratory. Congruent with the AFFRI research and development goal of "developing methods of rapidly assessing radiation exposure to assure appropriate medical treatment", the laboratory has begun a study of the pre- and post-radiation exposure proteome of murine buccal mucosa. Recent work has identified a transcription factor, nuclear factor KAPPA B (NF-KB) which induces the TNF- $\alpha$  encoding gene and activates the cyclooxygenase-2 (COX-2) pathway. At 24 hours post irradiation HIF-1 $\alpha$  and COX-2 protein levels were increased. In addition to its well established DNA-damage effects, ionizing radiation induces cell death, and radiation-induced activation of acid sphingomyelinases (ASMases) and the generation of ceramide. Ceramide is generated from sphingomyeline by the action of a neutral or ASMase or by de novo synthesis coordinated through the enzyme ceramidesynthase. Once generated, ceramide may serve as a second messenger molecule in signaling responses to physiologic or environmental stimuli, or it may be converted to a variety of structural or effector molecules. With a single dose of 3 Gy, there is activation of protein kinase B/AKT (PKB/AKT) signaling. Within minutes of irradiation, phosphorylation of the serine/threonine protein kinase PKB/AKT at serine-residue 473 appears. This activation of PKB/AKT contributes to inhibit glycogen synthase kinase-3 $\beta$  (GSK3 $\beta$ ), which has a clear inhibitory role in endothelial cell survival. [1809 Tan, J. 2006; 1719 Rodemann, H.P. 2007; ] This preliminary study describes the changes in murine buccal mucosa protein profiles when subjected to ionizing radiation in addition to those described above. [1716 Bonan, P.R. 2007; ] Buccal mucosa was chosen because of its known sensitivity to ionizing radiation and easy accessibility for study. The study was conducted under an approved protocol of the New York University School of Medicine Institutional Animal Care and Use Committee.

Fourteen swiss mice were anesthetized with intraperitoneal ketamine-xylazine (k/x, 80/10 mg/kg ip). Ten mice received 3 Gy to the head. Buccal mucosa was obtained fifteen minutes (5 mice) and thirty minutes (five mice) post exposure. Buccal mucosa from the four non-radiated mice were used as controls. All reagents were obtained from Sigma (St. Louis, MO) and used unmodified. All samples were placed in 1 mL of 100 mM ammonium bicarbonate buffer. Proteins were extracted from all the tissues in this buffer with high pressure (Barocycler, Pressure BioSciences, South Easton, MA). The samples were concentrated by lypholization to 200  $\mu$ L and divided into three components. One for trypsin digestion and LCMS analysis (bottoms up proteomics), one for HPLC protein separation and FTMS analysis (top-down proteomics) to identify post-translational modifications, and one for direct identification with MALDI mass spectrometry and bioinformatics analysis. Sinapic acid (5mg/mL) 0.3  $\mu$ L was pipetted onto a conductive MALDI plate, allowed to dry, and covered with an equal amount of sample. All fourteen samples were examined with MALDI mass spectrometry in linear mode. The findings were consistent across all four controls, 15 minute, and 30 minute samples, Figure 1. Cyano 4 hydroxy cinnamic acid (5mg/mL) 0.3  $\mu$ L was pipetted onto a conductive MALDI plate, allowed to dry, and covered with an equal amount of sample. All fourteen samples were examined with MALDI mass spectrometry in reflectron mode. The findings were consistent across all four controls, 15 minute, and 30 minute samples, Figure 6.

**Experimental**  
 Fourteen swiss mice were anesthetized with intraperitoneal ketamine-xylazine (k/x, 80/10 mg/kg ip). Ten mice received 3 Gy to the head. Buccal mucosa was obtained fifteen minutes (5 mice) and thirty minutes (five mice) post exposure. Buccal mucosa from the four non-radiated mice were used as controls. All reagents were obtained from Sigma (St. Louis, MO) and used unmodified. All samples were placed in 1 mL of 100 mM ammonium bicarbonate buffer. Proteins were extracted from all the tissues in this buffer with high pressure (Barocycler, Pressure BioSciences, South Easton, MA). The samples were concentrated by lypholization to 200  $\mu$ L and divided into three components. One for trypsin digestion and LCMS analysis (bottoms up proteomics), one for HPLC protein separation and FTMS analysis (top-down proteomics) to identify post-translational modifications, and one for direct identification with MALDI mass spectrometry and bioinformatics analysis. Sinapic acid (5mg/mL) 0.3  $\mu$ L was pipetted onto a conductive MALDI plate, allowed to dry, and covered with an equal amount of sample. All fourteen samples were examined with MALDI mass spectrometry in linear mode. The findings were consistent across all four controls, 15 minute, and 30 minute samples, Figure 1. Cyano 4 hydroxy cinnamic acid (5mg/mL) 0.3  $\mu$ L was pipetted onto a conductive MALDI plate, allowed to dry, and covered with an equal amount of sample. All fourteen samples were examined with MALDI mass spectrometry in reflectron mode. The findings were consistent across all four controls, 15 minute, and 30 minute samples, Figure 6.

The trypsin digest LCMS and HPLC prepared FTMS samples are the subject of another more extensive report.

**Results**  
 The linear mode MALDI MS experiment is shown in Figure 1. The lowest spectrum is a control sample. The middle spectrum is a 15 minute post 3 Gy sample. The upper spectrum is a 30 minute post 3 Gy sample. At 15 minutes post 3 Gy, a protein 63870.32 Da, DnaJ homolog subfamily C member 1 (DnaJ protein homolog MTJ1) [gi|2494160|sp|Q61712.1|DNJC1\_MOUSE[2494160] was demonstrated. DnaJ domains (J-domains) are associated with hsp70 heat-shock system and it is thought that this domain mediates the interaction. The putative structure of this domain is demonstrated in Figure 2. The structure of the DNA-binding domains is demonstrated in Figure 3. The telomeric DNA-binding site is shown in Figure 4. At 30 minutes post 3 Gy, a protein 72449.25 Da, mCG1463, isoform CRA\_b [Mus musculus] [gi|148683958|gb|EDL15905.1|[14868395] is identified. This protein is involved in transcriptional regulation. The structure is demonstrated in Figure 5. The NCBI database reveals these three peptide sequences and proteins they represent, Figure 7.

**Conclusion**  
 The protein and peptide profiles and bioinformatic identifications from the NCBI database are proof of principal that early changes post ionizing radiation can be easily and rapidly identified from buccal mucosa tissue scraping with mass spectrometry. This preclinical work heralds a clinical translation in head and neck cancer patients, for validation purposes. The long term aim is the identification of specific profiles that enable reliable associations with dose-exposure for biodosimetry.

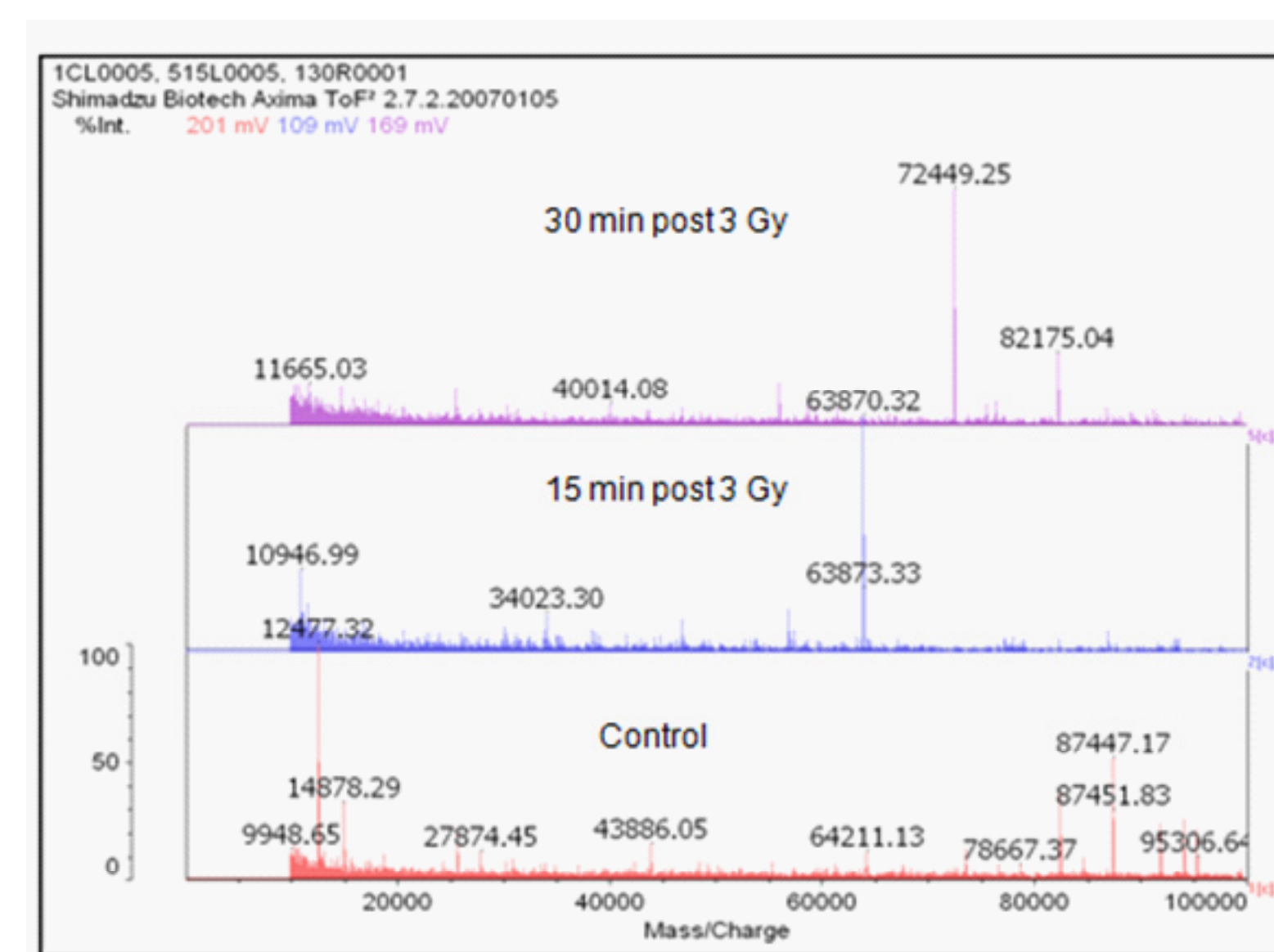


Figure 1. MALDI MS mass spectrometry, linear mode, control, 15 minutes post 3Gy, and 30 minutes post 3Gy. Note the proteins 63870.32 Da at 15 minutes, and 72449.25 at 30 minutes. These proteins can be identified with bioinformatics in the NCBI database.

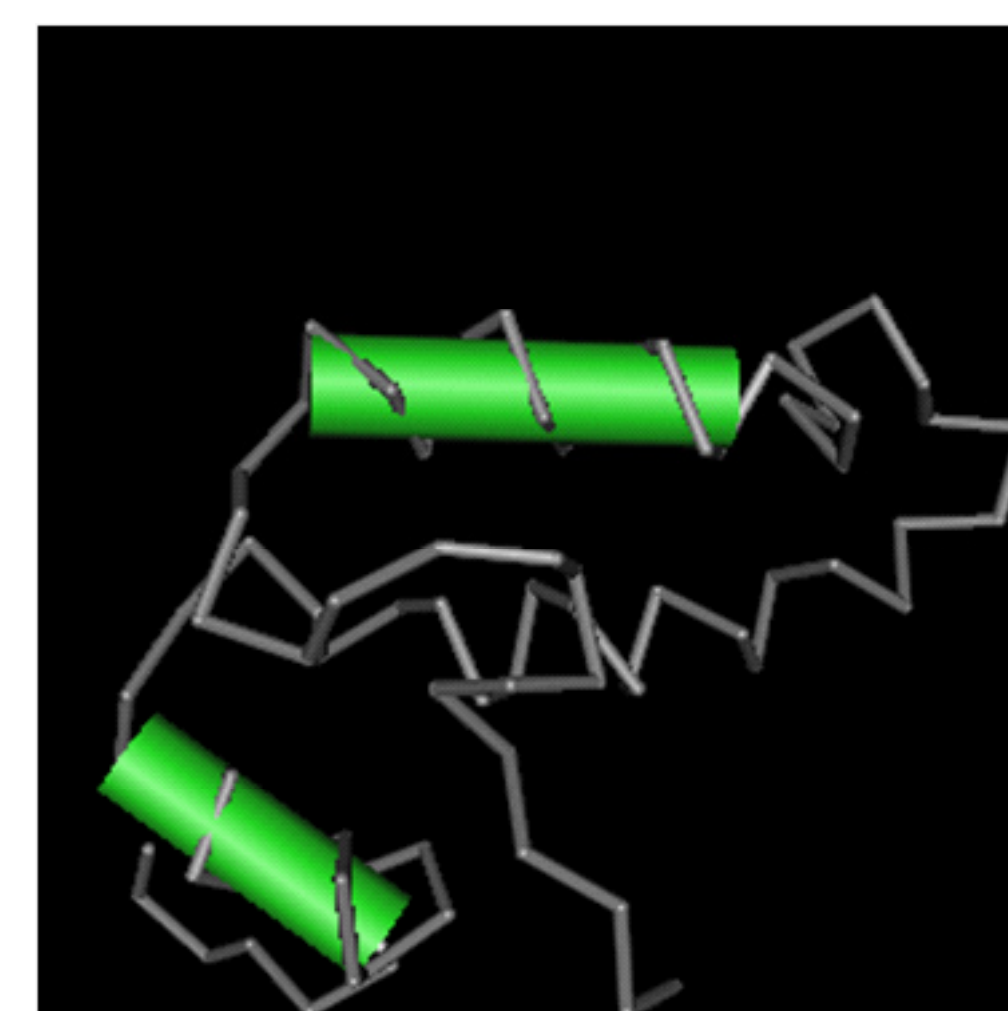


Figure 2. Structure of J-Domain.

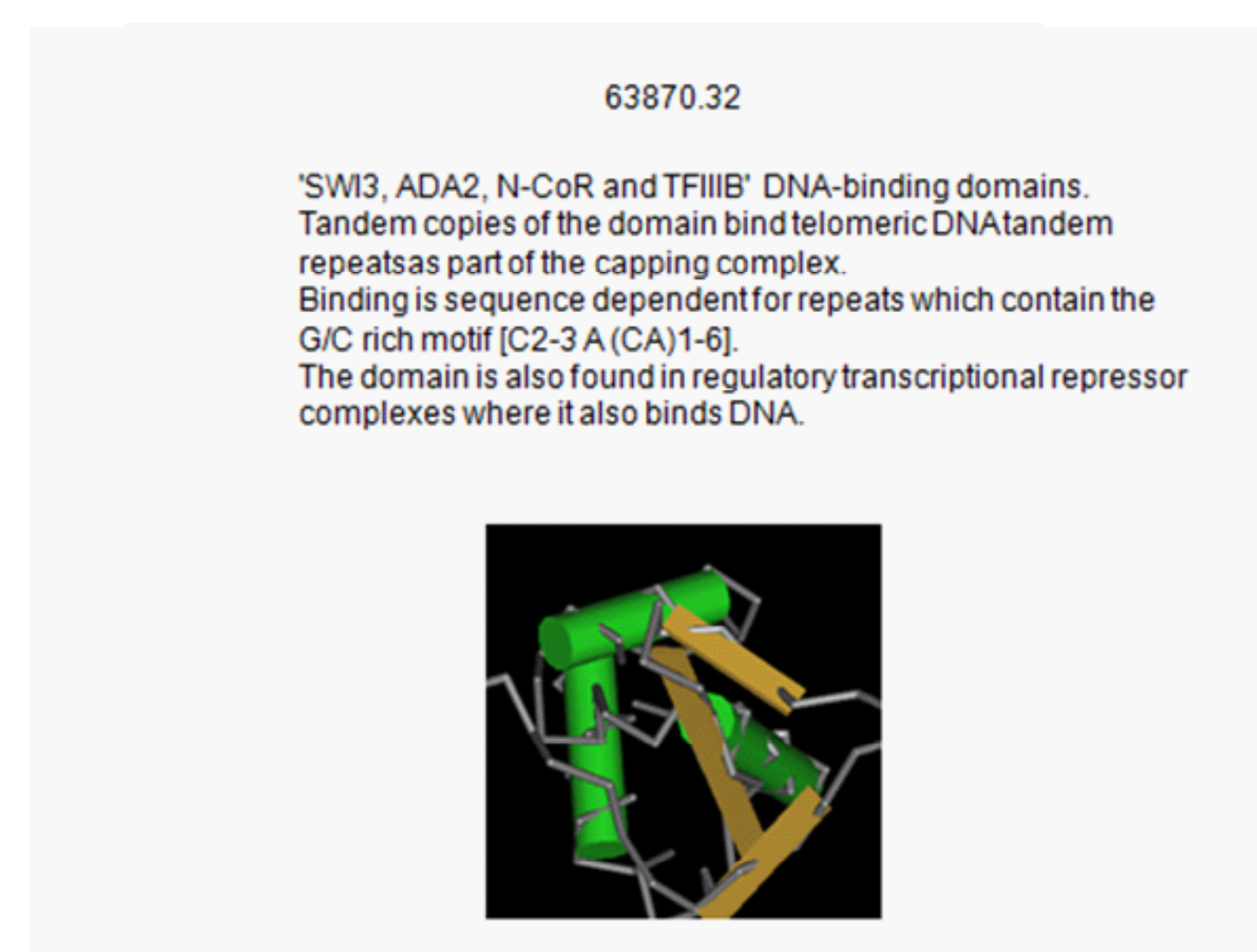


Figure 3. The SW13 and other DNA-binding domains.

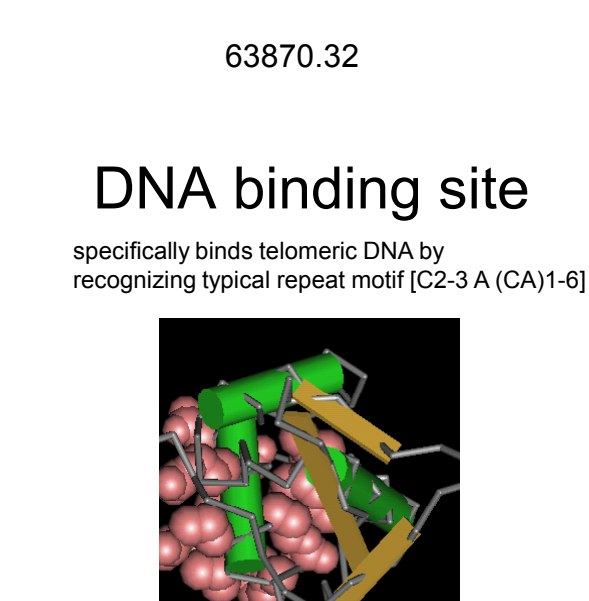


Figure 4. Telomeric DNA-binding site.

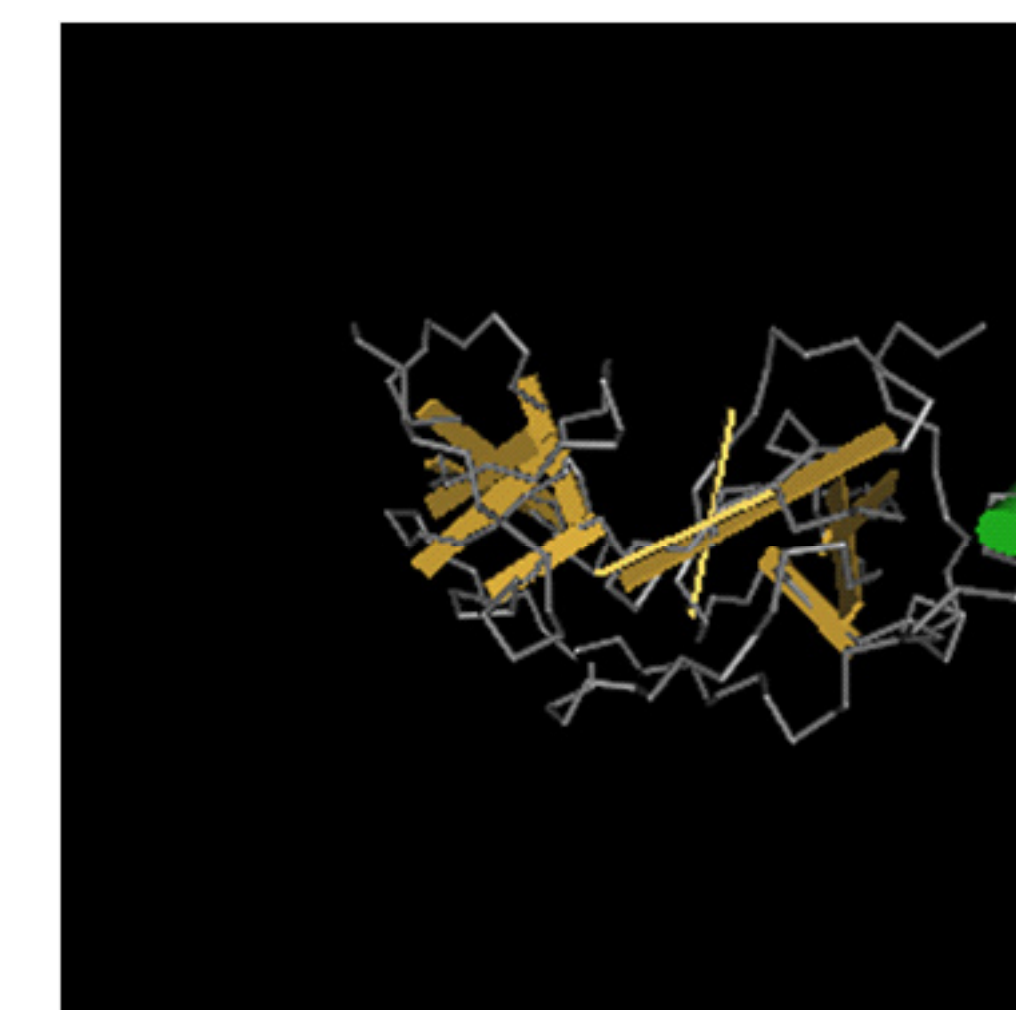


Figure 5. Structure of gi|148683958|.

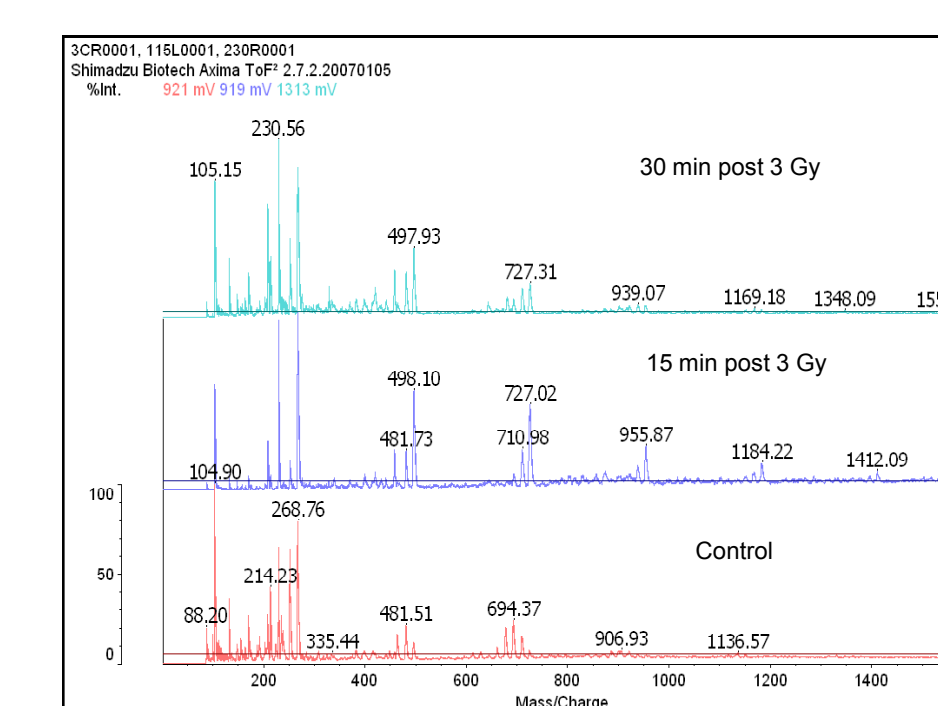


Figure 6. MALDI MS mass spectrometry, reflectron mode, control, 15 minutes post 3Gy, and 30 minutes post 3 Gy. Note 955.87 Da and 1184.12 Da peptides that appear at 15 minutes post 3Gy, and the 939.07 Da peptide that appears 30 minutes post 3 Gy.

Peptides at 15 and 30 min post 3 Gy			
15min	955.87	gi 45593111 novel protein (B230312A22R6) [Mus musculus]	mssgpkaped
	1184.12	gi 1333908 unnamed protein product [Mus musculus]	akdyrygygf
30 min	939.07	gi 168884609 transaldolase [Mus musculus]	mrvhqaets

Figure 7. Peptide amino acid sequences and pro-