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Date Printed: Jun 6, 2012

Experiment Safety Review Form
Review Number: PS-ESR-1-161-2012

PRINCIPAL INVESTIGATOR: Mark Chance, Mike Sullivan
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Project Title: General User Lab 1-161, Protein Sample Prep for Synchrotron Footprinting (At X28C)
Location(s): 0725
Area(s): 0725-FIRST-1-161
Proposed Start Date and Duration: 6/13/2011 - 1 years

SIGNATURES:

Principal Investigator: Mark Chance, Mike Sullivan	**Date: 6/22/2011**
Experiment Review Coordinator: Lori Stiegler	**Date: 6/22/2011**
Reviewer: Christopher Weilandics	**Date: 6/6/2011**
Reviewer: Frank Zafonte	**Date: 5/26/2011**
Reviewer: Deborah Bauer	**Date: 6/20/2011**
Reviewer: Brian Heneveld	**Date: 5/26/2011**
Approval: Lori Stiegler	**Date: 6/22/2011**
Review/Approval (ERC) Comments: 06/05/2012 4:32 PM No changes in scope or hazards. Renewed for one year 06/22/2011 4:48 PM Input PI signature approval date as per e-mail from Mike Sullivan 6/22. 06/22/2011 4:46 PM	
Walkthrough Signature:	**Date:**
Expiration Date (max 1 yr.): 6/22/2013	
FUA Change Required? No	
Fire Rescue Run Card Changes Required? No	
Has a NEPA Review been Performed for this Project? No	
Required Approvals (i.e., IACUC, IBC, etc.):	
Project Termination Acceptance Signature: **Comments:**	**Date:**

I. Define the Scope of the Work

A. Description

General Purpose/ Experimental Scope: To resolve solution dynamics of macromolecular interactions and conformational changes at single amino acid residue or nucleic acid base level. Hydroxyl radicals generated using X-ray radiation cause oxidative modifications in protein solutions, which are proteolytically digested and analyzed using mass spectrometry.

Equipment/ Apparatus: Invitrogen™ Cell4 *SureLock*™ Midi-Cell for SDS-PAGE analysis, pH meter for buffer preparation, Savant SpeedVac system for quick drying of protein samples, cold room storage (shared with lab 1-162), -80ºC freezer and refrigerators.

Apparatus operating conditions: All protein sample preparations, pipetting, pH measurements of stock buffer solutions, SDS-PAGE experiments are performed at room temperature (20 – 25 °C). Typically, protein samples are prepared in micro molar concentrations in 10mM Sodium phosphate pH 7.2 buffer and distributed in PCR tubes (200ul capacity) for X–ray exposure. 10mM Methionine Amide Hydrochloride solution is typically used to quench the hydroxyl radicals in solution after sample exposure at X28C. ~300ml 60mM Stock solution is made in phosphate buffer solution pH 7.0. The irradiated protein samples are brought back to 1-161. ~90% of the samples are shipped back to the general user. The remaining samples are used in the following way. Irradiated samples are proteolytically digested using trypsin, pepsin or V8 enzymes. pH is adjusted to either acidic or basic conditions using 20% 2-5ul trifluoroacetic acid (TFA) or 50mM Ammonium Carbonate/10mM Sodium phosphate buffers (50-200 ul) depending on the enzyme used. The protein and enzyme sample volume is usually in the range of 20- 200ul. Volumes of stock buffer solution used are 25-50 mL. The digestion is carried out at incubation temperature of 37 °C using heat bath generally for not more than 4 to 12 hrs. Sometimes, 5mM dithiothreitol (DTT) solution and 15mM iodoacetamide solution might be used before enzyme digestion. The digested protein samples are used in rm. 1-163 for LCMS analyses. For this purpose, HPLC solvents are prepared in the fume hood. Typically, 5-95% Acetonitrile is prepared in water with 0.1% Trifluoroacetic acid. The HPLC solvents are taken from the flammable cabinet in rm. 1-159. Also, extraction of membrane proteins is infrequently carried out using Methanol- chloroform- water extraction. SDS- PAGE is occasionally run to optimize and monitor digestion conditions. Protein solutions (in nanogram quantities) prepared in NuPAGE® LDS Sample Buffer (4x) are loaded in the **NuPAGE®** Novex® Midi Gel well. Generally, 750 ml of 1X NuPAGE® (MES) SDS Running Buffer is used for gel electrophoresis. After assembling the Cell4 *SureLock*™ Midi-Cell assembly and loading the protein samples the electrophoresis is performed for 40 minutes at a constant voltage of 200V and variable current of 160-120mA (according to the manufacturer published protocol). Protein samples are analyzed using silver staining method. Protein samples are usually stored at 4 - 8 °C or at -80 °C.

Infrequently (not more than 2-3 times a year), 500-750 ml, 10 mM Sodium Cacodylate buffer pH 7.2 is used for protein sample dialysis.

Note: All samples and reagents in samples are in microliter volume with a concentration range of micromolar to millimolar.

2. Metaloproteomics (at X3B) sample preparation

Purpose/ Experimental Scope: High throughput qualitative and quantitative analysis of transition metals in proteins sample solutions.

Equipment/ Apparatus: cold room storage (4 °C) and refrigerator (-80 °C)

Sample preparation: Typically, 100- 200 nanograms of protein sample solutions in SEC B3 + 5mM DTT

are distributed in Teflon plates and X-ray fluorescence data is collected at X3B analyzed to identify metalloproteins from proteins purified through structure genomics pipeline.

3. X3A/X29 Crystallography Users

Wet lab facility is the general requirement for the protein crystallography (PX) experiments at the synchrotron. Firstly, the cryoprotection of the protein crystal is one of the important steps in PX to acquire a good dataset. In order to search for suitable cryoprotectant one needs to screen several organic solvents. The basic principle is crystal has to be immersed in the cryoprotectant (organic solvent, oil, or salt based) before the flash freezing. Secondly, the lab is used to generate heavy atom derivatives. A quick-soak method has been applied to generate *de novo* heavy-atom phasing to solve new protein structures. The process involves soaking the crystals in the solutions containing halides of Br, I, etc. The heavy atom will diffuse into the crystal through solvent channels and bind to the protein at one or more sites. The initial phases are then deduced from differences between the diffraction data measured from native and derivative crystals. Lastly, crystallographers prepare the complex crystals by soaking the protein

crystal in inhibitor/substrate solutions. The wet lab is equipped with two microscopes for sample viewing located at room temperature and in cold room. One 2-liter capacity dewar and several smaller dewars are available for transporting liquid nitrogen and flash freezing of crystals. All the materials are brought by the users and they will take all solutions (and disposable plastics like pipet tips) back with them for disposal at their institute/university regulations.

Some users are scheduled for short period of X29 beam time when they come to use X3B. In such case, the samples for X29 are usually prepared in the same wet lab. The procedures are the same as described above.

4. X3B XAS Users

A small amount of protein samples (typically, 100-200 nanograms) are loaded in the sample holder slot and enclosed with kapton tape. The samples are sometime cryocooled using small amount of liquid nitrogen. No chemicals and solutions are used by the General Users if the samples are prepared at their respective institutions. Most of the users do not generate any waste and carry their samples after performing measurements on them back to their institutions. If wastes are to be generated contact with NSLS safety engineer will be required when review of their request to use lab form is reviewed. In addition, all chemicals and procedures are required to be listed on this form prior to use of this room.

5. General Users at NSLS

This laboratory is accessible to all users at the NSLS. As lab stewards, the Center for Synchrotron Bioscience (CSB) staff actively maintain and support the equipment owned by the CSB. To access to the lab, users are required to complete the laboratory request form posted on the entrance door to the lab and have one of the CSB staff review and sign the form. Equipment used by both staff and users includes an anaerobic glove box, several centrifuges of various sizes, microscopes, pH meters, thermally regulated blocks and baths, a cold room, a vortex, a vacuum oven, a sample microwave oven, a SpeedVac, a -80ºC freezer, refrigerators and a fume hood.

6. Glove Box

The TS-400 purchased from Vacuum/Atmospheres Company, Hawthorne, California is an inert atmosphere chamber mainly used for sample preparation and mounting. It has two vacuum chambers for equipment and sample loading into the main chamber. The re-generation process requires a 265 cu ft cylinder of 5% hydrogen and the balance nitrogen. The box has a constant supply of nitrogen from the NSLS boil off system. This gas is used to replace the atmosphere inside the box as the gloves are inserted and when the antechambers are refilled during the pump purge process.

Equipment manuals or procedures that are controlled documents:

N/A

B. Human Performance Factors

N/A

C. Waste Minimization/Pollution Prevention

Smallest quantities necessary are used.

D. Materials Used /Waste Generated

Materials Used	Disposal Method	Amount per Use	Amount per Year	Comments
Proteins (non-hazardous)	Sanitary	0.50 mg	5.00 mg	
Enzymes (trypsin, pepsin, V8)	Sanitary	10.00 ul	0.10 mg	
Ammonium bicarbonate (buffer salts)	Sanitary	50.00 ul	2.50 mg	
sodium phosphate dibasic (buffer salts)	Sanitary	1.70 ul	0.80 mg	
Trifluoroacetic acid	Hazardous	50.00 ul	1.80 ml	Present in protein solution in trace amounts and further used at room 1-163 and disposed of through HPLC combined waste or sent back to user
Dithiothreitol (5 mM)	Hazardous	100.00 ul	500.00 ul	
Acetonitrile	Hazardous	3.00 ml	900.00 ml	Present in protein solution in trace amounts and further used at room 1-163 and disposed of through HPLC combined waste or sent back to user
Silver staining	Hazardous	300.00 ml	300.00 ml	Disposed of immediately as non-rad haz waste
Biorad reagent (commassie blue stain)	Hazardous	150.00 ml	150.00 ml	
Chloroform	Fugitive	400.00 ul	4.00 ml	
Methanol	Fugitive	400.00 ul	4.00 ml	
Methionine amide hydrochloride	Fugitive	3.00 mg	15.00 mg	
NaBr		2.00 mg	10.00 mg	
NaI		2.00 mg	10.00 mg	
10 mM Sodium cacodylate (buffer salts)	Hazardous	600.00 ml	2.00 ltr	Disposed of immediately as non-rad haz waste using 90-day area

II. Identify and Analyze Hazards Associated with the Work

The following hazards were identified:

Physical Hazards:

- Cryogens (any substance or device capable of producing temperatures <= 170K) (Areas: 0725-FIRST-1-161, 0725-FIRST-1-161)
- Compressed gases (lecture bottles, cylinders, gas lines) (Areas: 0725-FIRST-1-161, 0725-FIRST-1-161)
- Flammable liquids (Areas: 0725-FIRST-1-161, 0725-FIRST-1-161)

Chemical Hazards:

- Carcinogens (Areas: 0725-FIRST-1-161, 0725-FIRST-1-161)
- Reproductive toxins (Areas: 0725-FIRST-1-161, 0725-FIRST-1-161)
- Corrosives
- Flammable liquids (Areas: 0725-FIRST-1-161, 0725-FIRST-1-161)
- Toxic metals (e.g., As, Ba, Be, Cd, Cr, Hg, Pb, Se, Ag) (Areas: 0725-FIRST-1-161, 0725-FIRST-1-161)

Ionizing and Non-ionizing Radiation Hazards:

- None

Biological Hazards:

- None

Offsite Work:

- None

Other Issues (Security, Notifications, Community, etc.):

- None

Significant Environmental Aspects

- Any amount of hazardous waste generation (Area: 0725-FIRST-1-161)

III. Develop and Implement Hazard Controls and Assess Risk

A. Physical Hazards, Tasks and Controls

Hazard, Default Controls, Task Specific Info	Risk Level
Hazard: **Cryogens (any substance or device capable of producing temperatures <= 170K)** **Default Controls:** General Requirements: • Evaluate location oxygen deficiency • Store/transport only in approved containers (i.e. DOT/ASME or BNL LESHC) • Never pour from above chest level • PPE: Long Sleeve Shirt (or Lab Coat), long pants (or skirt covering ankles) and closed shoes Pressurized transfer to open (vented) container; Or-Pouring > 5 liter volumes of LN2 between open containers: • Face shield along with either Safety Glasses (w/side shields) or Goggles • Gloves (Cryo or Heavy Leather) Pouring small (5 liters or less) volumes of LN2 between open containers: • Safety Goggles (face shield recommended if possible) • Gloves (Cryo or Heavy Leather) Work with samples immersed in LN2 in small (~1 liter) dewars: • Use Tongs (tools) to manipulate/handle cryogenic samples (do not touch with gloves)• Use insulated non-absorbent gloves with dexterity (cotton/nylon gloves under disposable nitrile gloves) • Safety Goggles	**Negligible (0-20)**
Hazard: **Compressed gases (lecture bottles, cylinders, gas lines)** **Default Controls:** • Any systems >15psi must be SME Approved • Transport cylinders using a cylinder cart • Secure cylinders to a fixed object/wall • Use regulator, hoses, and components compatible with gas • Use hoses and clamps rated for maximum regulator output or use pressure relief device • Wear safety glasses with side shields when installing/removing/or adjusting regulator • Label piping/tubing	**Negligible (0-20)**
Hazard: **Flammable liquids** **Default Controls:** As for chemicals, plus Store large quantities in Flam. cabinets as required	**Negligible (0-20)**

B. Chemical Hazards, Tasks and Controls

Hazard, Default Controls, Task Specific Info	Risk Level
Hazard: **Carcinogens** **Default Controls:** Use hazardous chemicals controls plus: Establish and post a "designated area" where used Note location on fire run card and hazard info. placard.	**Negligible (0-20)**

Exposure monitoring to assure that action levels not exceeded Establish procedures for work area cleanup and decontamination **Task Specific Info:** Safety glasses with side shields or goggles, nitrile gloves (splash protection only), lab coat	
Hazard: **Reproductive toxins** **Default Controls:** Use hazardous chemicals controls plus: Establish and post a "designated area" where used Note location on fire run card and hazard info. placard for storage of solids, greater than 40 pounds; liquids, greater than 5 gallons; gases, greater than 10 pounds Exposure monitoring, determined by SHSD Rep, to assure that action levels not exceeded Prior to decommissioning, establish procedures for sampling and decontamination **Task Specific Info:** Safety glasses with side shields or goggles, nitrile gloves (splash protection only), lab coat	**Negligible (0-20)**
Hazard: **Corrosives** **Default Controls:** Use hazardous chemicals controls plus: - Unobstructed access to Emergency Eyewash and Shower **Task Specific Info:** Safety glasses with side shields or goggles, nitrile gloves (splash protection only), lab coat Keep lab door open for access to eye wash/shower	**Negligible (0-20)**
Hazard: **Flammable liquids** **Default Controls:** Use hazardous chemicals controls. Review large quantity storage with Fire Protection Engineer Note location on fire run card and hazard info. placard for storage of solids, greater than 40 pounds; liquids, greater than 5 gallons; gases, greater than 10 pounds	**Negligible (0-20)**
Hazard: **Toxic metals (e.g., As, Ba, Be, Cd, Cr, Hg, Pb, Se, Ag)** **Default Controls:** As for chemicals, plus need for SHSD and OMC monitoring and surveillance must be evaluated BURF for Beryllium operation	**Negligible (0-20)**

C. Environmental Hazards, Tasks and Controls (include on/off site transportation and products/services)

Hazard, Default Controls, Task Specific Info	Risk Level
Hazard: **Any amount of hazardous waste generation** **Default Controls:** Engineering Controls • Waste will be accumulated in chemically compatible containers that appropriately contain/protect the waste. • Waste containers will be closed in a tray (secondary containment) in the Satellite Accumulation Area (SAA).	**Negligible (0-20)**

Administrative Controls • All hazardous waste containers will have a (red) “Hazardous Waste Label” that has the generator’s name and the chemical contents (trade name/formula not acceptable). • All waste will be accumulated in closed containers and kept in an established and posted SAA until ready for transfer to the 90-Day Haz Waste Area for pick-up by Waste Management. • For pick-up by Waste management, complete the Nonradioactive Haz Waste Control Form and consult the 90-Day Area Manager to gain access/transfer the waste to the 90Day Area. Training: Hazardous Waste Gen. (HP-RCRIGEN3). PPE: When handling waste materials follow PPE requirements specified for the specific materials. Comply with the SBMS Subject Area: “Hazardous Waste Management”.	

D. Radiation Hazards, Tasks and Controls
None

E. Biological Hazards, Tasks and Controls
None

F. Offsite Work Hazards, Tasks and Controls
None

G. Other Issues (Security, Notifications to Other Organizations, Community Involvement, etc.)
None

H. Recommended Exposure Monitoring

- None

Description or comments:

I. EPHA Determination

Chemical Name	Quantity (lbs, gal)	Location (Bldg/Room#)

IV. Perform Work Within Controls

A. Recommended Training and Medical Surveillance Summary

- Laboratory Standard (HP-IND-220)
- Cryogen Safety (HP-OSH-025)
- Hazardous Waste Generator (HP-RCRIGEN3)
- Compressed Gas Safety (TQ-COMPGAS1)

B. Personnel Training, Qualification, and Authorization List

Employee/Guest Name	Life/Guest#	Dept	Required Training Course(s)	Signed
Mike Sullivan	S5438	LU	Cryogen Safety (HP-OSH-025) [EXPIRES: NEVER] Compressed Gas Safety (TQ-COMPGAS1) [EXPIRES: 5/17/2015] Laboratory Standard (HP-IND-220) [EXPIRES: 5/31/2013] Hazardous Waste Generator (HP-RCRIGEN3) [EXPIRES: 7/22/2012]	
Rhijuta D'Mello	J6432	LU	Cryogen Safety (HP-OSH-025) [EXPIRES: NEVER] Compressed Gas Safety (TQ-COMPGAS1) [EXPIRES: 3/4/2013] Laboratory Standard (HP-IND-220) [EXPIRES: 11/3/2012]	

			Hazardous Waste Generator (HP-RCRIGEN3) [EXPIRES: 11/9/2012]	
Erik Farquhar	Y5735	LU	Cryogen Safety (HP-OSH-025) [EXPIRES: NEVER] Compressed Gas Safety (TQ-COMPGAS1) [EXPIRES: 8/9/2013] Laboratory Standard (HP-IND-220) [EXPIRES: 8/9/2012] Hazardous Waste Generator (HP-RCRIGEN3) [UNASSIGNED: EXPIRES: 11/18/2012]	

C. Emergency Procedures

Follow the NSLS Local Emergency Plan. Call the Control Room for any incidents or spills. Spill control supplies are located outside room 1-166.

D. Transportation

None

E. Logistical Interactions

None

F. Termination/Decommissioning

All chemicals and equipment will be transferred or dispositioned upon transfer of lab steward or change in use of room.

V. Provide Feedback

None

VI. Attachments

Attached Files:

06-03-10 LEEF 161&163.pdf