Structural Studies of Prions by X-ray Footprinting at NSLS-II

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NSLS-II User Proposal

Covalent modification of human Prions by OH radicals

• User:
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  Funded by National Institutes of Health

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  Beamline: XFP (17-BM)
Prions Diseases in General

- **Human**: Kuru, Creutzfeldt-Jakob Disease (CJD), Gerstmann-Sträussler-Scheinker Disease (GSS), Fatal Insomnia (FI)
- **Animal**: Scrapie, Bovine Spongiform Encephalopathy (BSE), Chronic Wasting Disease (CWD)

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**Normal cellular mouse monomeric prion protein (PrPC)**

- α-helix B
- α-helix C

**Model of hamster pathogenic aggregated prion protein (PrPSc)**

- Cofactor

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James et al., 1997, Billeter et al., 1997

Grovenman, JBC 2014
Model for Prion Infectivity

Transmission by ingestion or intravenous injection

Prion replication cycle

PrP^Sc

nucleation

breakage

breakage into multiple new seeds

elongation, conformational change

infection

PrPC

sensitive

resistant

modification by •OH
Goal: Insight Into the Structure of Prion Protein Aggregates Using X-ray Footprinting

• Advantages of synchrotron source compare to the other techniques:
  • Fast, in water (physiological conditions), without other chemicals like in Fenton, reproducible

• Our goal:
  • Understanding of the causation of prion aggregation
  • Facilitate an effective search for drugs that inhibit replication of human prions
Schematic of Experiment

- Multiple Sample Holder
- Detector for Beam Alignment
- Shutter
- Beam Pipe (Vacuum)
- Motorized Slide
- Sample (in PCR tube)
- x-ray beam
High Throughput Apparatus
Biosafety Levels are Specified by CDC

- **BSL1**: agents that do not cause disease, e.g. Baker’s yeast. Work on open benchtop with standard safety procedures. Most biological studies at BNL are BSL1.

- **BSL2**: agents that are difficult to transmit by aerosols, e.g. HIV. More training, limited access to lab, laminar flow hood needed for procedures that can generate aerosols. Several BSL2 studies at BNL: transgenic plants in the field, BoTox structure.

- **BSL3**: agents that cause lethal diseases for which treatments are available, e.g. *M. tuberculosis*, West Nile virus. Lots more engineering controls and training. **No facility available at BNL.**

- **BSL4**: lethal agents for which no vaccines or treatment is available, e.g. Marburg virus, Ebola virus. Even more controls and training than BSL3. **Prohibited by DOE.**
Risk Analysis

• No history of occupational illness related to prion research
• Prions transmitted mainly through ingestion and injection (not inhalation).
• Samples are sealed the entire time that they are at BNL
• Samples arrive and leave in triple containment
• Experimental apparatus operated only when hutch is interlocked
• Camera on sample will show if sample opens prior to reentry into hutch
• Samples secured when not in use
• Sample inventory and Chain of Custody control implemented
• Experiment estimated to take 4hrs to complete
Review and Approval of Project

- NSLS II PASS proposal submitted May 2017 for fall cycle beamtime at XFP (GU) includes BSL-2 work
- Institutional Biosafety Committee (IBC) proposal submitted
- NSLS-II Biohazard Risk Screening completed
- Detailed operating procedure written
  - Based on previous successful protocol for work with mouse prions,
  - Developed with PIs (Jiri Safar, Marislova Kacirova, CWRU), NSLS-II safety staff, and IBC
- NSLS II Safety Approval Form submitted by PI with associated documentation attached
- IBC review and approval
- NSLS-II approval, pending
- IRMC approval, pending
Questions?