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

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NSLS-II User Proposal

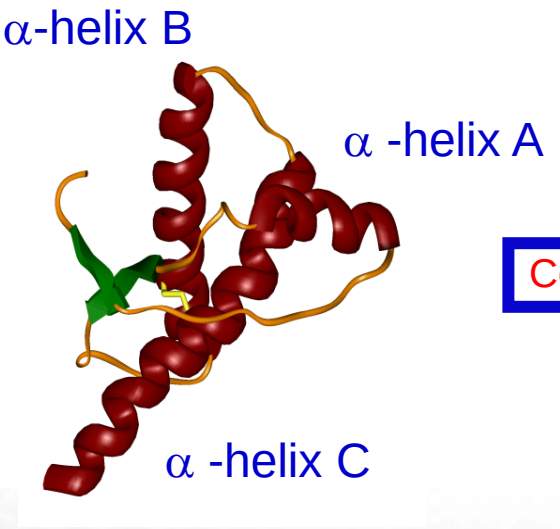
Covalent modification of human Prions by OH radicals

- User:
  - Dr. Jiri Safar, M.D.
  - Department of Pathology
  - Case Western Reserve University
  - Director, National Prion Disease Pathology Surveillance Center
  - Funded by National Institutes of Health
- NSLS-II Beamline Scientist
  - Dr. Jennifer Bohon
  - 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)

Normal cellular mouse monomeric prion protein (PrP^{C})

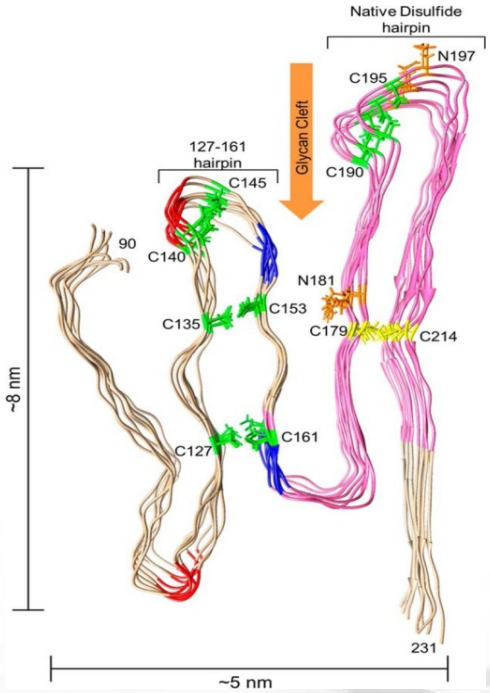


James et al., 1997, Billeter et al., 1997

Cofactor

?

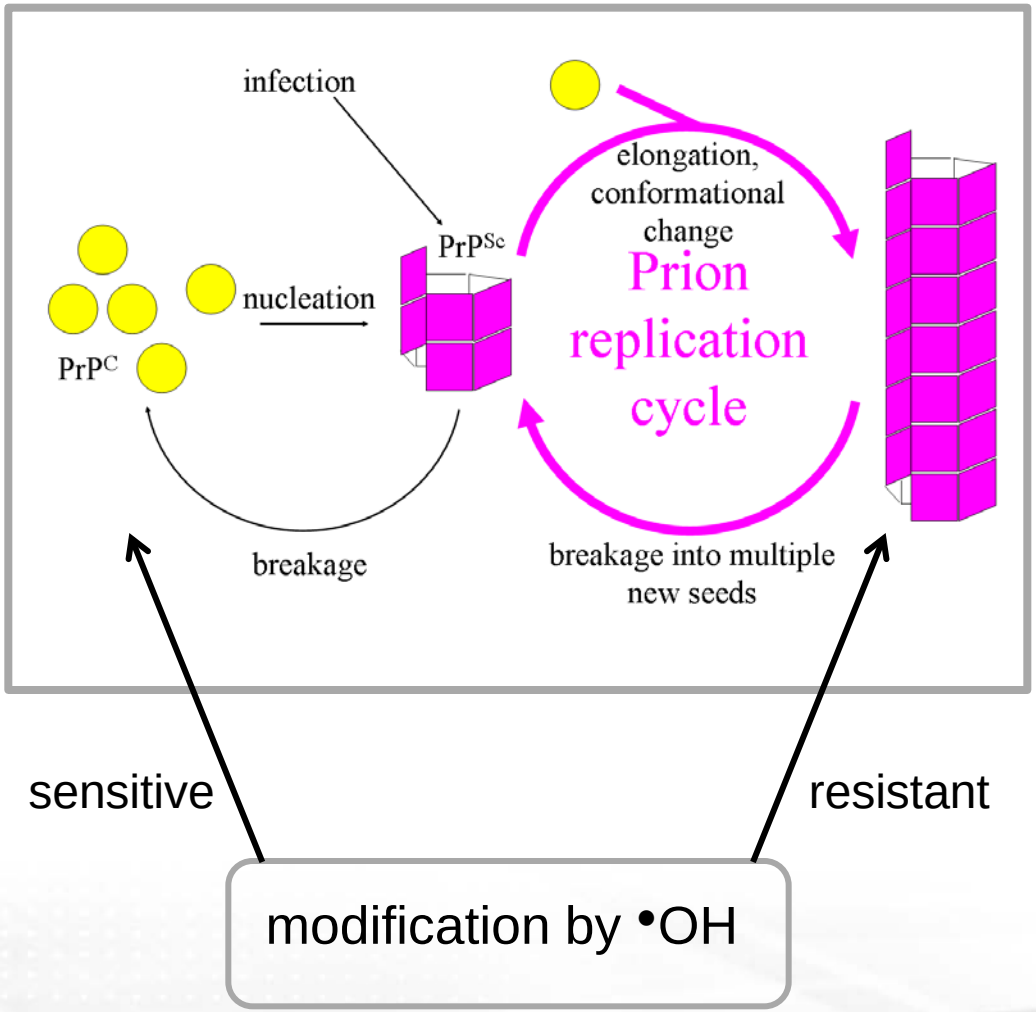
Model of hamster aggregated prion protein (PrP^{Sc})



Groveman, JBC 2014

Model for Prion Infectivity

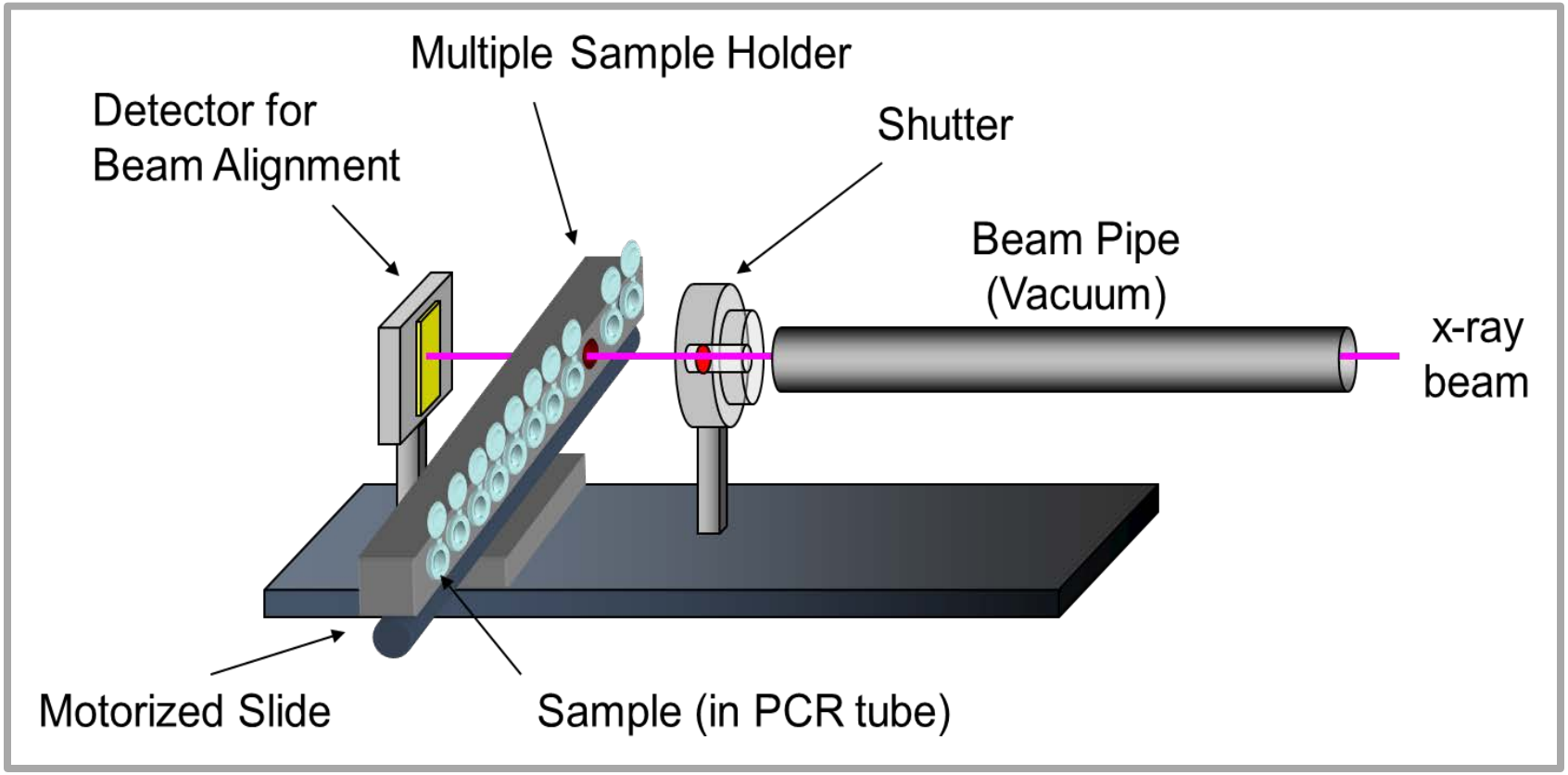
Transmission by ingestion or intravenous injection



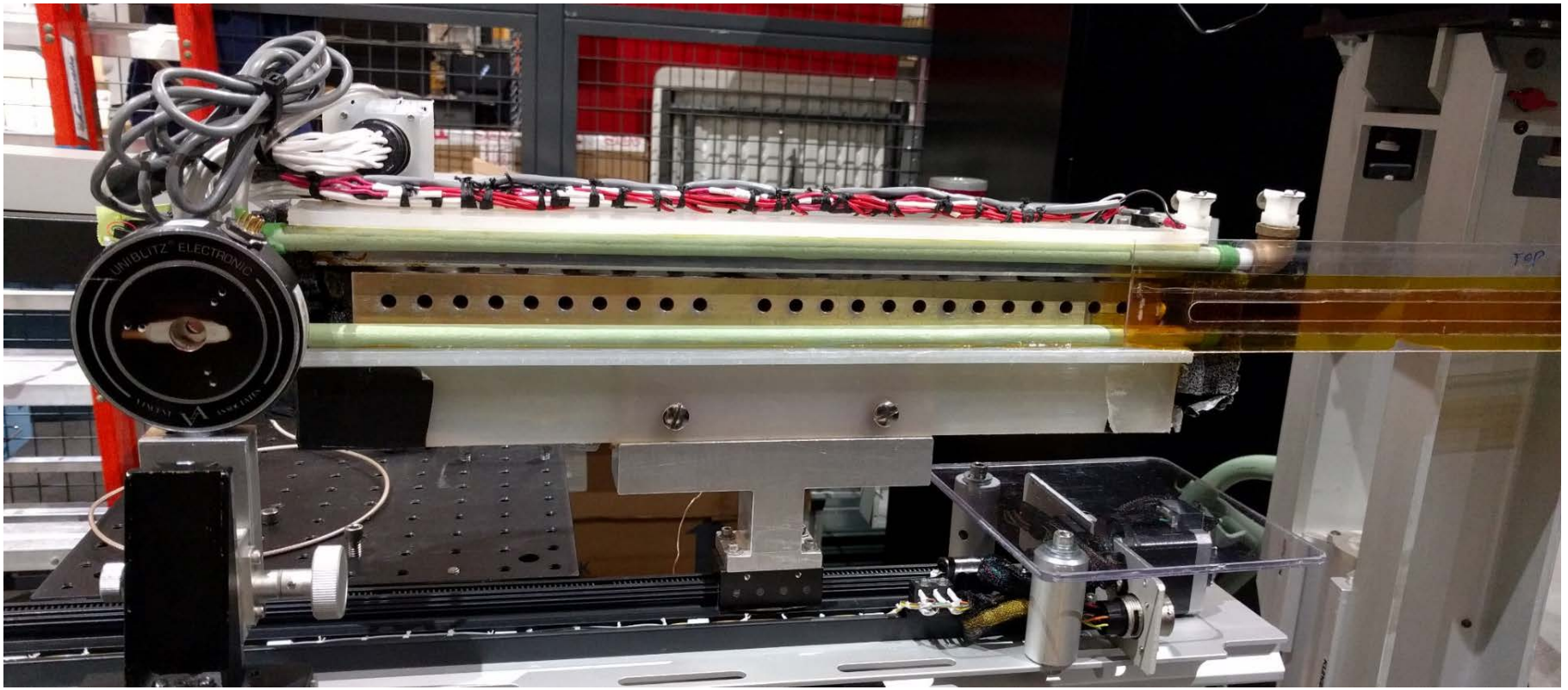
Goal: Insight Into the Structure of Prion Protein Aggregates Using X-ray Footprinting

- **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



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?