During the next half-century the world's elderly population (those 60 years and older) will grow from 600 million to nearly 2 billion (1). This population will then comprise 15% of the world's population, an increase of 7% from the year 2000. The societal causes of mortality are gradually shifting from infectious and parasitic diseases to chronic diseases, increasing the societal burden of disability due to aging and age-related diseases. As an example, the risk of Alzheimer's disease (AD) increases 14-fold from age 65 to 85, affecting as many as 42% of individuals over the age of 85. Despite much recent progress, our understanding of the cellular - mechanisms that underlie age-related neurodegeneration remains incomplete.

Highlight the overall significance of the project in a way that is understandable by a general audience. Here, the author is focusing on the world's aging population, a societal issue that most readers can recognize as being important.

Use proper citations where appropriate.

The mechanism of neurodegenerative disorders such as Alzheimer's, Parkinson's, Huntington's and prion diseases all involve protein misfolding and deposition of insoluble protein aggregates (2). Prion diseases are characterized by the aberrant folding of a glycolipid-anchored protein, termed PrPC, into a self-propagating aggregated conformer, termed PrPSc. However, unlike other neurodegenerative disorders, prion diseases are infectious and readily transmissible to animal and cultured cells. Inoculation of mice with prion isolates results in defined clinical disease with associated symptoms and histopathologies. Similarly, exposure of cultured neuroblastoma to prions results in the intracellular accumulation of infectious protein aggregates. The availability of robust cell and mouse models makes prion disease an experimentally tractable protein misfolding disorder and provides a unique opportunity to investigate the downstream consequences of protein aggregate accumulation within a cell.

Describe the specific focus of the project. Here, the author describes the specific neurodegenerative disease that will be studied and its importance.

Although the role of PrPSc as the causative agent in prion disease has been proven, the exact molecular and cellular mechanism of its formation and the ensuing neurodegeneration remain under investigation. The expression of PrPC is required for pathogenesis, indicating that prion disorders are not loss of function diseases (3). It is widely accepted that that neuoronal cell death is caused by the toxic properties of a subpopulation of PrPSc conformers. Two general mechanisms of toxicity have been proposed for PrPSc aggregates (4, 5). The first proposes that PrPSc interacts with specific cell-surface receptors, initiating an apoptotic cascade. The second proposes that the intracellular accumulation of prion aggregates compromise the normal cellular quality control mechanisms of the cell, ultimately leading to cell death. Support for the latter hypothesis comes from experiments that link PrPSc accumulation to inhibition of both the ubiquitin-proteasome and autophagy-lysosome systems (6). However, the global consequence of this inhibition on proteome catabolism and turnover has not been investigated.

Describe previous work in this subject area. Here, the author explains what is known about this disease, and what remains to be studied. Notice that as the proposal progresses, it is starting to become more specific and detailed.

Recently, methods have been developed for analyzing protein turnover rates with a cell using mass spectrometry-based proteomics (9). This approach can be used to analyze the turnover rates of thousands of proteins in multiple tissues and cell types. In this project, we are proposing to use this approach to evaluate the effects of prion infection on protein turnover rates within a cell.

If some preliminary work has already been done on this project (by the author or others), this should be briefly mentioned. Here, the author is describing a method that has already been developed that will be used in this project.

The specific aims of this project are as follows:

Describe specifically what will be done in this project, including specific techniques and methodologies. What kind of data will be produced or what product will be created? Here the author is describing these details in terms of two "specific aims." Highlighting "specific aims" is one common strategy used in STEM-related proposals. However, describing "specific aims" is not an absolute requirement for this proposal. The methodology or approach can be described in other formats as well.

<u>1. Analyze the effect of prion accumulation on proteome dynamics in cultured cells</u>
Two different cell lines (N2a and GT1) will be infected with the Rocky Mountain Labs (RML) mouse-adapted scrapie prion strain. Infected cells will be labeled with 15N and proteome turnover will be analyzed using the proteomic strategy described above. Subsequent bioinformatic analysis will identify cellular catabolic pathways that are impacted by prion infection. To validate the results, prion infected cells will be treated with compounds that induce the clearance of intracellular prion aggregates and the turnover analysis will be repeated.

<u>2. Analyze the effect of prion accumulation on proteome dynamics in infected mice</u>
We will assess the effect of neurodegeneration on proteome turnover in mouse models of prion disease. Mice will be inoculated with the RML prion strain and brain tissues will be collected at different time points post inoculation, along with age-matched controls. Turnover profiles of the brain will be assessed using the approaches described above and altered genes will be identified. Comparison of results with cell culture data obtained in the first specific aim will highlight pathways that are impacted by intracellular aggregate formation in both in vitro and in vivo models of prion disease.

Together, these two will provide a proteome-wide compendium of changes in protein half-lives associated with prion disease and highlight the role of specific catabolic pathways in prion-induced pathogenesis. These results may help identify new therapeutic approaches targeted against prion diseases.

It is always a good idea to describe the overall impact of the project. Here, the author is concluding the proposal by reiterating what will be learned by this project, and the societal impact it may have.

1. Kinsella K, Velkoff VA. The demographics of aging. Aging Clin Exp Res. 2002;14(3):159-69.
2. Prusiner SB. Shattuck lecture--neurodegenerative diseases and prions. N Engl J Med. 2001;344(20):1516-26.
3. Büeler H, Aguzzi A, Sailer A, Greiner R-A, Autenried P, Aguet M, Weissmann C. Mice devoid of PrP are resistant to scrapie. Cell. 1993;73:1339-47.
4. Crozet C, Beranger F, Lehmann S. Cellular pathogenesis in prion diseases. Vet Res. 2008;39(4):44.
5. Aguzzi A, Sigurdson C, Heikenwaelder M. Molecular mechanisms of prion pathogenesis. Annu Rev Pathol. 2008;3:11-40.

A bibliography can be included in any standard format.