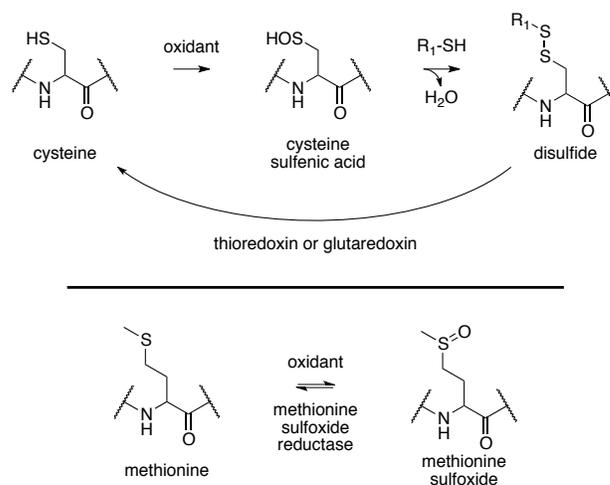


## HHMI Summer Research Scholar Proposal–Inhibition of Methionine Sulfoxide Reductases by Organic Electrophiles

Perturbations in intracellular redox status can alter the lifespan and fitness of organisms from many different biological kingdoms.<sup>1</sup> To counteract the toxic effects of elevated oxidant levels, organisms have developed elaborate defense mechanisms that clear these molecules and repair oxidized protein targets. Two amino acids bearing sulfur atoms—cysteine and methionine—are the most common targets of oxidants in proteins.<sup>2</sup> Therefore, many organisms possess reductases that repair oxidized sulfur atoms in the proteins targeted by oxidants. With cysteine, repair of oxidation is carried out by the glutaredoxin and thioredoxin enzymes,<sup>3</sup> whereas, with methionine, the reduction reaction is facilitated by methionine sulfoxide reductases (Fig. 1).<sup>4</sup>



**Figure 1. Oxidation and Reduction of Cysteine and Methionine.**

In the baker's yeast *Saccharomyces cerevisiae*, there are three distinct reductases that repair oxidized methionine. Using protein cross-linkers, we have found that two of these proteins—Mxr1 and Ykg9—are targets of organic electrophiles, undergoing intramolecular cross-linking in yeast treated with a thiol-reactive protein cross-linker. To follow up on these preliminary experiments, the HHMI summer research scholar selected for this project will explore the hypothesis that **organic electrophiles inactivate Mxr1 and Ykg9, resulting in increased levels of oxidized methionine in baker's yeast.** Specifically, we will:

**1. Determine which residues form cross-links in yeast methionine sulfoxide reductases following exposure to protein cross-linkers.** To reduce methionine sulfoxide, Ykg9 and Mxr1 use redox-active cysteine residues in their active sites. The reactivity of their active site cysteines likely renders these proteins susceptible to modification by electrophiles (e.g., with the cross-linkers we have studied earlier). Therefore, we will use site-directed mutagenesis to mutate active site cysteines within Mxr1 and Ykg9 to alanine, a non-reactive amino acid. Subsequently, we will determine which cysteines become modified to form intramolecular cross-links in yeast expressing these mutant proteins that are exposed to a variety of cross-linkers.

**2. Explore the effect of organic electrophiles on the enzymatic activities of Mxr1 and Ykg9.** If the active site cysteines are modified by electrophiles, as we would expect, then it is likely that Mxr1 and Ykg9 are inactivated under these conditions. Therefore, we will express and purify Mxr1 and Ykg9. Subsequent activity assays will be conducted with Mxr1 and Ykg9 that has been treated with electrophiles, according to previously published methods,<sup>5</sup> to determine the extent of inhibition.

**3. Monitor overall levels of oxidized methionine in the yeast proteome following exposure of cultures to electrophiles.** If Mxr1 and Ykg9 are inhibited by electrophiles in cells, one would predict that there would be an increase in overall levels of methionine sulfoxide. To this end, we will use an antibody that recognizes oxidized methionine to detect any alterations in cells treated with electrophiles, performing Western blots for this analysis.

With the proposed project, we will continue employing protein cross-linkers to explore the structure and functions of various proteins. Moreover, by exploring the influence of electrophiles on the activity of methionine sulfoxide reductases, we will further develop our understanding of protein damage caused by this class of toxins.

## References.

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