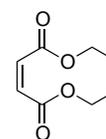


Project Title: Activation of the Heat Shock Response by Diethyl Maleate and Structurally Related Molecules

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Research Proposal: Maintaining proper protein folding within cells requires active surveillance and considerable investment of energy throughout an organism's lifetime.¹ In humans, impaired protein folding has been closely linked with a variety of age-related disorders, including many neurodegenerative diseases.¹ As a result, developing pharmacological strategies to restore the protein folding capabilities of a cell to a normal level is an area of active investigation. A potential way of ensuring proper protein folding homeostasis is through increasing the levels of molecular chaperones, a group of proteins that facilitate protein folding.² Many of these chaperones are heat shock proteins that are commonly induced by the transcription factor heat shock factor 1 (Hsf1) upon a cell's exposure to protein-damaging stresses.

A number of known chemical Hsf1 activators can be classified as organic electrophiles that damage target proteins to promote heat shock response activation.² Such molecules have commonly been studied individually, where detailed structure-function studies with analogous molecules have not been performed. Consequently, the data that suggest how an electrophile's chemical and physical properties—its hydrophobicity, thiol adduct stability, and number of electrophilic centers—alter heat shock response activation are limited. The focus of this HHMI SEER application is to determine which of an electrophile's physical and chemical properties enhance expression of heat shock proteins in mammalian cells for a specific class of organic electrophiles related to diethyl maleate (Fig. 1). With previous support from HHMI, students and I have



diethyl maleate

Figure 1. Structure of diethyl maleate.

characterized the cell death responses and protein damage carried out by this class of molecules, identifying the protein cross-linker diethyl acetylenedicarboxylate (Fig. 2).^{3,4}

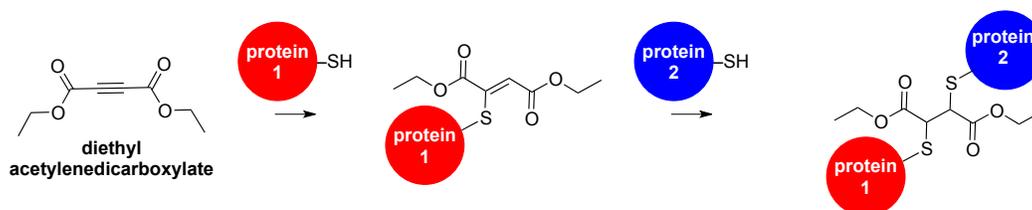


Figure 2. Cross-linking of proteins by diethyl acetylenedicarboxylate.

Here, we will **perform structure-activity studies with various diethyl maleate analogs, including diethyl acetylenedicarboxylate, to determine how structural differences in these molecules influence activation of the heat shock response.** The selected SEER mentor and incoming students will monitor how diethyl maleate analogs influence the expression of an Hsf1-regulated reporter gene and endogenous heat shock protein gene expression (using RT-PCR and Western blots) in human cells. By relating our previous work on electrophile-protein interactions with their potency as heat shock response activators, we will **identify which structural attributes among the electrophiles studied are common predictors for Hsf1 activation.** Such information will aid in answering a largely unresolved question within this field and establish the groundwork for additional studies on similar Hsf1 activators in the future.

References.

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