Despite recent advances in the new therapies for acute myeloid leukemia (AML), the outcome for most patients remains poor. Although targeted therapies have recently been approved for patients with AML, most patients do not respond to these treatments and when responses do occur, remissions are brief. Thus, it remains critical to investigate novel ways to target AML using strategies that are rooted in a mechanistic understanding of the disease and the infrastructure AML cells use to survive. One such system is the human mitochondrial matrix ClpXP protease. It is comprised of the ClpX unfoldase and the ClpP protease which function to maintain the integrity of oxidative phosphorylation by degrading damaged/misfolded respiratory chain proteins. Notably, ClpXP is overexpressed in a subset of AML patients and inhibiting ClpXP kills AML cells with the high levels of the protease, while sparing normal cells. To date specific and potent inhibitors of ClpXP have not been developed. This proposal aims to develop a novel mechanism of inhibition that operates based on a recently discovered allosteric pathway in bacterial ClpP enzymes. I will identify a conformational switch in human ClpP that, upon mutagenesis, leads to a catalytically inactive structure that can be reactivated through the binding of small-molecule activators. I will use a tightly integrated structural and biochemical investigation, involving HDX-MS and functional assays, to fully characterize this inhibition mechanism in human ClpP. Given that ClpP is overexpressed in a broad range of cancers, there is exciting potential for this work to have translational value beyond AML.