A Directed Evolution Approach to Engineering Caspase Specificities
Maureen Hill, Derek MacPherson and Jeanne A. Hardy, PhD

Engineered proteases have potential as both biological therapeutics and molecular tools. For example, proteases in which the active sites have been reengineered to recognize one unique sequence that is present in a particular molecular target can be used to specifically cleave that target and thus eliminate its function from the cell. This approach is currently under investigation by a number of biotech companies such as Catalyst Biosciences. On the other hand, engineering allosteric sites in proteases provides a means of orthogonal control of an individual protease from within a large protease family. The ability to selectively inactivate one member of a protease family allows us to determine the function of individual family members. Because of the promise of these and other uses for engineered proteases, we have developed a method for engineering both active and allosteric sites in proteases.

Our laboratory focuses on caspase proteases, which play important regulatory roles in apoptotic cell death and in inflammation. They are amongst the most specific of all proteases, and are therefore excellent targets for engineering specificity. Unfortunately, because they are heterotetramers with very adaptable active sites, they are not amenable to traditional engineering approaches (e.g. phage display) or to rational design. Our chalk talk will describe our development of a GFP-based reporter of caspase activity that has enabled us to perform a directed evolution screen and evolve caspase function. We will present data on several new, evolved versions of caspase-7, in which the specificity has been converted to that of caspase-6. Our structural analysis helps to define substrate specificity across the caspase family, and underscores why rational design is insufficient for altering the specificity and why directed evolution is required.