A coupled protein and probe engineering approach for selective inhibition and activity-based probe labeling of the caspases

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Caspases are cysteine proteases that play essential roles in apoptosis and inflammation. Unfortunately, their highly conserved active sites and overlapping substrate specificities make it difficult to use inhibitors or activity-based probes to study the function, activation, localization, and regulation of individual members of this family. Here we describe a strategy to engineer a caspase to contain a latent nucleophile that can be targeted by a probe containing a suitably placed electrophile, thereby allowing specific, irreversible inhibition and labeling of only the engineered protease. To accomplish this, we have identified a non-conserved residue on the small subunit of all caspases that is near the substrate-binding pocket and that can be mutated to a non-catalytic cysteine residue. We demonstrate that an active-site probe containing an irreversible binding acrylamide electrophile can specifically target this cysteine residue. Here we validate the approach using the apoptotic mediator, caspase-8, and the inflammasome effector, caspase-1. We show that the engineered enzymes are functionally identical to the wild-type enzymes and that the approach allows specific inhibition and direct imaging of the engineered targets in cells. Therefore, this method can be used to image localization and activation as well as the functional contributions of individual caspase proteases to the process of cell death or inflammation.

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