CHEMICAL BIOLOGY

# Engineering to find function

**Protein engineering and small-molecule probe design allows the study of individual members of large protease families.**

Members of large, biologically and medically important protease classes, including the caspases and matrix metalloproteinases (MMPs), contain highly conserved active sites and overlapping substrate preferences. This makes studying the function of individual proteases within a biological context difficult using traditional inhibitor design.

Matthew Bogyo of Stanford University School of Medicine and his colleagues have come up with a clever way around this problem. They find a suitable residue near the active site of either an MMP or a caspase and mutate it to a cysteine. The engineered cysteine can be covalently modified with a probe that binds to the active site and inhibits protease activity.

Bogyo and his team applied the strategy to two MMPs, MMP12 and MT1-MMP (Morell *et al.*, 2013), and two caspases, caspase-8 and caspase-1 (Xiao *et al.*, 2013). They used previous structural or sequence conservation knowledge to guide their choice of a residue that could be modified to a cysteine without affecting substrate specificity.

To design a small-molecule probe to selectively inhibit the engineered MMPs, they began with a broad-spectrum MMP inhibitor and decorated it with an electrophile to bind the mutant cysteine, a hydroxamate group to bind the zinc in the active site and a Cy5 fluorescent tag. For the caspases, they started with a peptide-based inhibitor and modified it with an acrylamide electrophile to bind the mutant cysteine, an aldehyde electrophile to bind the active-site cysteine and a carboxyfluorescein fluorescent tag.

The cysteine-mutant MMPs and caspases were functional in mammalian cells, in which they were specifically labeled by the designed fluorescent probes. Bogyo's team further demonstrated that expressing the engineered MT1-MMP in zebrafish rescued a craniofacial morphogenesis phenotype induced by MT1-MMPa/b knockdown. Additionally, they showed that macrophage cells stably expressing the mutant caspase-1 infected with *Salmonella* experienced pyroptotic death (associated with the antimicrobial response during inflammation), which requires functional caspase-1.

The strategy is general and will help researchers dissect the similar but distinctive functions of individual MMPs and caspases.

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RESEARCH PAPERS

Morell, M. *et al.* Coupling protein engineering with probe design to inhibit and image matrix metalloproteinases with controlled specificity. *J. Am. Chem. Soc.* **135**, 9139–9148 (2013).

Xiao, J. *et al.* A coupled protein and probe engineering approach for selective inhibition and activity-based probe labeling of the caspases. *J. Am. Chem. Soc.* **135**, 9130–9138 (2013).