In search of perfection - the phenomenon of unnatural amino acids

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Proteolysis is one of the most fundamental and ancient biochemical reactions, orchestrated by enzymes known as proteases. These enzymes act as molecular gatekeepers: the "good guys" safeguarding cellular homeostasis through processes such as protein quality control, apoptosis, blood coagulation, and signal transduction. Yet, proteases can also become "bad guys," driving pathological mechanisms underlying cancer, diabetes, coagulopathies, inflammation, infections, and neurodegenerative disorders. Unsurprisingly, they represent a major focus in drug discovery, with an estimated 5–10% of all pharmaceutical targets involving proteases.

Despite their importance, studying proteases remains a formidable challenge. Many proteases share overlapping substrate preferences for natural amino acids, as well as similar localization, making it exceedingly difficult to distinguish individual enzyme families using conventional chemical tools. This lack of specificity not only hinders our ability to map proteolytic networks but also obstructs the discovery of selective biomarkers and therapeutics. A striking example is the caspase family of cysteine proteases, whose intricate regulation of apoptosis and other pathways cannot be fully resolved with existing methods.

As more proteases are implicated in human disease, there is an urgent need for next-generation chemical tools capable of monitoring their activity with precision. Activity-based probes (ABPs), which bind only to active enzyme forms, provide a powerful solution. Our group has pioneered the development of ultrasensitive substrates, inhibitors, and ABPs for key protease families using a breakthrough strategy: the Hybrid Combinatorial Substrate Library (HyCoSuL). By incorporating unnatural amino acids into peptide scaffolds, HyCoSuL dramatically expands the landscape of substrate specificity, enabling unprecedented resolution in probing proteolytic activity. Beyond diagnostics, this technology also opens new horizons in rational drug design. For example, protease inhibitors designed with these principles underlie modern antiviral therapies such as Paxlovid, illustrating the transformative potential of HyCoSuL-guided strategies for developing the next generation of precision medicines.

1. Drag & Salvesen, *Nature Reviews Drug Discovery* **2010**, 9, 690
2. Kasperkiewicz et al. *Proc. Natl. Acad. Sci. U S A.* **2014**, 111, 2518
3. Poręba et al. *Cell Chemical Biology* **2016**, 23, 1023
4. Rut et al, *Antiviral Research* **2017**, 139, 88
6. Poreba et al., *Nature Protocols*, **2017**, *12*, 2189
5. Kasperkiewicz et al., *Journal of the American Chemical Society* **2017**, 139, 10115
4. Rut et al, *Nature Chemical Biology*, **2020**
5. Rut et al. *Chemical Science*, **2020**
6. Rut et al., *Science Advances*, **2020**