# Using activity-based probes to understand α-amylase substrate specificity

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α-amylases are useful industrial tools for the commercial processing of starches and related carbohydrates. These glycosyl hydrolases, of the GH13 class, break down a-1,4 bonds and are essential to the production of high-fructose corn syrups, modern detergents, and starch-derived biofuels. The discovery and engineering of GH13 enzymes which can withstand the harsh conditions often utilised in these processes is of high market value. These harsh conditions often involve extreme pH and temperature, and of particular interest in this work, a lack of calcium. Many GH13 enzymes are calcium-dependent.

In this work, we present the application of activity based probes based on an a-1,4-linked disaccharide for the study of GH13 activities. An epoxide group is installed to trap the covalent intermediate. These probes are used to profile a panel of a-amylases against a range of different conditions to understand more about their applicability to different industrial processes. These profiling assays aim to demonstrate the ease of using activity based probes to identify suitable enzymes for a particular set of process conditions.

The development of a-1,6 branched probes is also presented. These branched probes are used to profile GH13 a-amylases in human gut bacteria which may accommodate a branch in a particular position in the substrate grove. These insights are used to explain the ability of some of these bacteria to grow on complex carbohydrates.

Bibliographic references:

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