Trinity College Dublin
Coláiste na Tríonóide, Baile Átha Cliath
The University of Dublin

Latent active site probes to study endogenous enzyme activity

Overview

Deubiquitinating enzymes (DUBs) are known to have numerous important interactions with the ubiquitin cascade and their dysregulation is associated with several diseases, including cancer and neurodegeneration. They are an important class of enzyme, and activity-based probes have been developed as an effective strategy to study them.

Existing activity-based probes that target the active site of these enzymes work *via* nucleophilic mechanisms.

The team at Trinity College Dublin have developed latent ubiquitin-based probes that target DUBs *via* a site selective, photoinitiated radical mechanism.

This approach differs from existing photocross linking probes as it requires a free active site cysteine. In contrast to existing cysteine reactive probes, control over the timing of the enzyme-probe reaction is possible as the alkene warhead is completely inert under ambient conditions, even upon probe binding.

The probe's reactivity has been demonstrated against recombinant DUBs and to capture endogenous DUB activity in cell lysate. This allows more finely resolved investigations of DUBs

Applications

Our technology provides a novel method for assaying deubiquitinating enzyme (DUB) activity in complex environments which are key for testing of inhibitors and examining the function of DUBs. As with previous probes, the potency of irreversible inhibitors can be assessed. Crucially, the labelling strategy also allows reversible inhibitors to be evaluated more effectively than existing solution. Our method of probing reversible inhibitors is significantly more effective than using existing solutions, where the probe can displace a reversable inhibitor and accumulate over time. One of the major uses of the current probes is to evaluate inhibitors as DUBs are key drug targets. For example, the DUB USP7 is a key target in cancer therapy and of the key proteases in SARS-CoV-2.

The technology we describe is the first instance of a truly latent activity-based probe of any type and so can open up new avenues for basic science and drug discovery.

Technology and Patent Status

A priority patent had been filed at the UK patent office; GB2003936.8

2020 Publication in Chemical Science
https://doi.org/10.1039/C9SC05258E

The opportunity

Trinity College is seeking to collaborate and/or licence the technology to pharmaceutical company for development and commercialisation.

Market

Drug Discovery, Reagents

IP Status

Priority Application filed

GB2003936.8

Opportunity

Research collaboration,

Available to license

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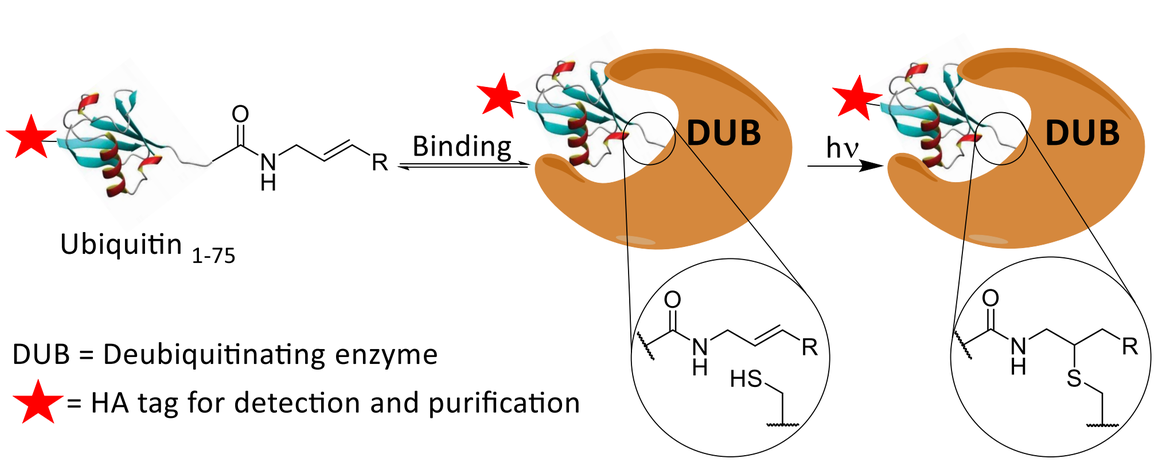

Proposed covalent bond formation of probes to DUBs via a thiol-ene reaction following the establishment of a binding equilibrium.