Installation of Electrophiles onto the C-terminus of recombinant ubiquitin and ubiquitin-like proteins

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Ubiquitin and related ubiquitin-like proteins (Ubls) influence a variety of cellular pathways including protein degradation and response to viral infections. The chemical interrogation of these complex enzymatic cascades relies on the use of tailored activity-based probes (ABPs). We report the preparation of ABPs for ubiquitin and a range of Ubls, including NEDD8, SUMO2, ISG15 and UFM1 by selective acyl hydrazide modification. Acyl hydrazides of Ubls are readily accessible by direct hydrazinolysis of Ubl-intein fusions. The suppressed pKa and superior nucleophilicity of the acyl hydrazides enables their selective modification at acidic pH with carboxylic acid anhydrides. The modification proceeds rapidly and efficiently, and does not require chromatographic purification or refolding of the probes. We modified Ubl–NHNH2 with various thiol-reactive electrophiles that couple selectively with E2s and DUBs. The ease of modification enables the rapid generation and screening of ubiquitin probes with various C-terminal truncations and warheads for the selection of the most suitable combination for a given E2 or DUB.