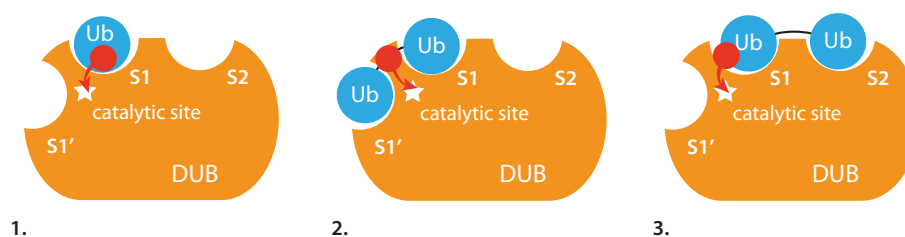


ACTIVITY-BASED DUB PROBES

Activity-based probes for deubiquitinases (DUBs) are based on ubiquitin with a C-terminal warhead designed to react with the active site cysteine residue present in most DUBs.

UbiQ offers a selection of activity-based probes (ABPs) with various *N*-terminal tags (see p2-3). They enable the researcher to crystallize, identify and validate dozens of DUBs and ubiquitin like proteases enzymes as potential targets involved in protein deubiquitination.



Three generations of DUB probes:

- 1st generation of DUB probes target the S1 pocket of a DUB and have contributed greatly to our understanding of DUBs.
- 2nd generation of DUB probes target the S1 and S1' pockets. These are di-ubiquitin-based probes with an electrophilic group between the two ubiquitins.
- 3rd generation of DUB probes target the S1 and S2 pocket and are useful tools to identify DUBs that rely on S2 site interactions to fine tune their activity.

applications

- structural biology studies of DUB-Ub complexes ^{1a, 5a, 5b}
- activity based protein (DUB) profiling
 - » *phenotypic, inhibitor affinity, selectivity, specificity profiling*^{1, 2a, 2b}
- chemical proteomics
 - » *pull down, purification, western blotting, MS, NMR*
- enhancement of poly-Ub chain accumulation, by inhibiting hydrolysis of poly-Ub chains on substrate proteins

our experience

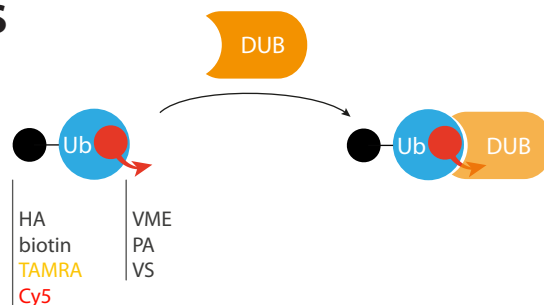
UbiQ introduced the propargylamide (PA) electrophile-, di-ubiquitin- and fluorescently labeled DUB probes.

These tools enabled:

- crystallization of several DUBs, including:
 - » *MINDY1, UCH-L5, USP4, and CCHF DUB : using our PA electrophile*^{7, 6a, 6b, 1a}
 - » *SARS PL PRO DUB : with our di-ubiquitin probe*
- target validation and identification of DUBs in infectious diseases : using the fluorescently labeled DUB probes. EU ITN Upstream project university of Glasgow and GSK.
- 15 tailor-made DUB probes for various customers

ACTIVITY-BASED DUB PROBES

UbiQ's wide selection of activity-based DUB probes consists of various electrophiles, substrates and N-terminal tags. In addition there is the option to order tailor-made activity based probes.



electrophiles

UbiQ offers three different electrophiles: propargylamide (PA or Prg), vinylmethyl ester (VME) and vinylsulfone (VS). All are irreversible DUB inhibitors and together these target all major DUB families.

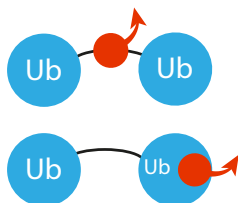
	PA / Prg	VME	VS
Target DUBs	OTU, USP, UCH, MINDY* and Machado-Josephin Domain ^{1**}	USP and UCH	USP and UCH
Linkage	irreversible, covalent linkage can be cleaved by acid treatment	irreversible	irreversible

* a recently discovered DUB family see ref 7

** according to a proteomics study the PA probes also target DUBs of the Machado-Josephin Domain family.¹

substrate context³

It is now possible to investigate your DUB with a high level of substrate context. UbiQ offers probes with ubiquitin, sumo2, and distal and proximal ubiquitin context.



NEW distal and proximal ubiquitin context

label DUBs in a linkage specific way

- target the S1, S1' and S2 DUB pockets
- closely mimic the native di-ubiquitin structure
- all di-ubiquitin linkages available

N-terminal tags

TAMRA ^{2a}	Cy5 ^{2a}	Biotin ²	HA ²
fluorescence detection		affinity tag	
<ul style="list-style-type: none"> • fast (in-gel), sensitive, and distinct read-out • no background labelling by cross reactivity as seen in immunoblots 		<ul style="list-style-type: none"> • allows pull-down • detection by western-blotting 	
• exc 550 nm, abs 590 nm	• exc 625 nm, abs 670 nm	• strongest known non-covalent interaction	• influenza epitope tag

literature

- (a) Ekkebus et al. *J Am Chem Soc* **2013**, *135*, 2867. (b) Sommer et al. *Bioorg Med Chem* **2013**, *21*, 2511.
 - (a) de Jong et al. *ChemBioChem* **2012**, *13*, 2251. (b) Altun et al. *Chem Biol* **2011**, *18*, 1401.
 - (a) Mulder & El Oualid et al. *ChemBioChem* **2014**, *15*, 946. (b) El Oualid et al. *Angew. Chem. Int. Ed.* **2010**, *49*, 10149.
 - (a) Misaghi et al. *J. Biol. Chem.* **2005**, *280*, 1512. (b) Galardy et al. *Methods in Enzymology* **2005**, *399*, 120.
 - (a) Borodovsky et al. *Chemistry and Biology* **2002**, *9*, 1149. (b) Borodovsky et al. *EMBO J.* **2001**, *20*, 5187.
 - (a) Clerici, M., et al. *Nat. Commun.* 2014, *5*, 5399. (b) Sahtoe, D.D. et al. *Mol. Cell* 2015, *57*, 887
 - (a) Rehman, A., et al. *Mol Cell.* 2016, *63*, 1. (b) Bekes, M. et al. *Mol. Cell* 2016, *62*, 572
- **For a complete list of references please we refer to the product group overview document.**

CATALOGUE OF ACTIVITY-BASED DUB PROBES

Substrate	Tag	Electrophile	Code	Name
UbiQ-probes explorer panels				
Ub	HA, Biotin, TAMRA, Cy5	VME/PA	UbiQ-L02	DUB probe explorer panel 10 probes = 5 x VME and 5 x PA, tags: HA, Biotin, TAMRA and Cy5
diUb	-	VME	UbiQ-L04	di-ubiquitin probe explorer panel with DUB activity-based probes based on K6, 11, 27, 29, 33, 48, 63

1st generation DUB probes: target the S1 pocket

Ub	-	VS	UbiQ-108	Ub-VS
Ub	-	PA (Prg)	UbiQ-057	Ub-PA
Ub	HA	PA (Prg)	UbiQ-078	HA-Ahx-Ahx-Ub-PA
Ub	Biotin	PA (Prg)	UbiQ-076	Biotin-Ahx-Ub-PA
Ub	TAMRA	PA (Prg)	UbiQ-058	TAMRA-Ub-PA
Ub	Cy5	PA (Prg)	UbiQ-072	Cy5-Ub-PA
Ub	-	VME	UbiQ-005	Ub-VME
Ub	HA	VME	UbiQ-035	HA-Ahx-Ahx-Ub-VME
Ub	Biotin	VME	UbiQ-054	Biotin-Ahx-Ub-VME
Ub	TAMRA	VME	UbiQ-050	TAMRA-Ub-VME
Ub	Cy5	VME	UbiQ-071	Cy5-Ub-VME

2nd generation DUB probes: target the S1 and S1' pockets

K6 diUb	-	VME	UbiQ-081	K6 diUb-VME
K11 diUb	-	VME	UbiQ-082	K11 diUb-VME
K27 diUb	-	VME	UbiQ-083	K27 diUb-VME
K29 diUb	-	VME	UbiQ-084	K29 diUb-VME
K33 diUb	-	VME	UbiQ-085	K33 diUb-VME
K48 diUb	-	VME	UbiQ-086	K48 diUb-VME
K63 diUb	-	VME	UbiQ-087	K63 diUb-VME

3rd generation DUB probes: target the S1 and S2 pockets

K63 diUb	-	PA	UbiQ-114	K63 diUb-PA
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tailor made

A series of proprietary techniques give us structural control on all aspects of our reagents, enabling us to construct reagents that are beyond the reach of any currently available alternative approaches.

Please contact us for more information.