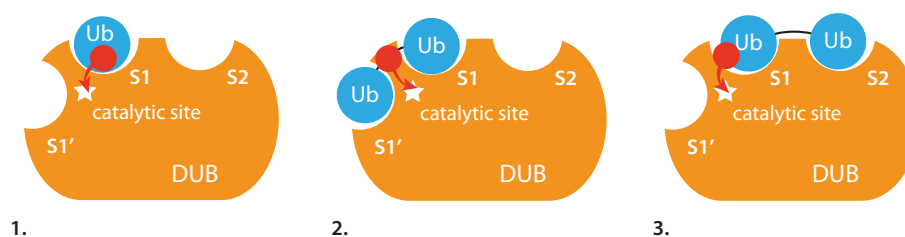


## ACTIVITY-BASED DUB PROBES

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 <sup>1a, 5a, 5b</sup>
- activity based protein (DUB) profiling
  - » *phenotypic, inhibitor affinity, selectivity, specificity profiling*<sup>1, 2a, 2b</sup>
- 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

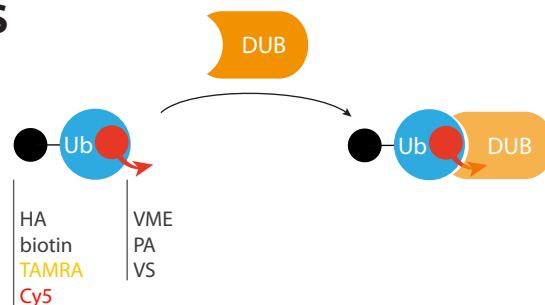
UbiQ introduced the propargylamide (PA) electrophile-, di-ubiquitin- and fluorescently labeled DUB probes.

These tools enabled:

- crystallization of several DUBs, including:
  - » *MINDY1, UCH-L5, USP4, and CCHF DUB : using our PA electrophile*<sup>7, 6a, 6b, 1a</sup>
  - » *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.

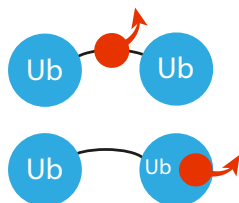
|                    | PA / Prg   | VME          | VS           |
|--------------------|--|--------------|--------------|
| <b>Target DUBs</b> | OTU, USP, UCH, MINDY* and Machado-Josephin Domain <sup>1**</sup> | USP and UCH  | USP and UCH  |
| <b>Linkage</b>     | irreversible, covalent linkage can be 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.<sup>1</sup>

### substrate context<sup>3</sup>

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 <sup>2a</sup>   | Cy5 <sup>2a</sup>        | Biotin <sup>2</sup>   | HA <sup>2</sup>         |
|---|--------------------------|---|-------------------------|
| fluorescence detection  |                          | affinity tag  |                         |
| <ul style="list-style-type: none"> <li>• fast (in-gel), sensitive, and distinct read-out</li> <li>• no background labelling by cross reactivity as seen in immunoblots</li> </ul> |                          | <ul style="list-style-type: none"> <li>• allows pull-down</li> <li>• detection by western-blotting</li> </ul> |                         |
| • 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   |
|------------------------------------|------------------------|--------------|----------|--|
| <b>UbiQ-probes explorer panels</b> |                        |              |          |  |
| Ub                                 | HA, Biotin, TAMRA, Cy5 | VME/PA       | UbiQ-L02 | DUB probe explorer panel<br>10 probes = 5 x VME and 5 x PA,<br>tags: HA, Biotin, TAMRA and Cy5             |
| diUb                               | -                      | VME          | UbiQ-L04 | di-ubiquitin probe explorer panel<br>with DUB activity-based probes based on<br>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 |
|----------|---|----|----------|-------------|

### 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.