UbiQ Probes

TRIPLE E PROBES
ACTIVITY-BASED PROBES FOR E1-E2-E3 ENZYMES

With UbiQ's revolutionary activity-based E1-E2-E3 probe it has become possible to crystallize, identify or validate dozens of enzymes, or potential targets, involved in protein ubiquitination. The probe is processed in a native way by the E1-E2-E3 cascade. In addition to this it has the option of reacting with the active site cysteine residues in an irreversible way, thus trapping the enzymes in the ubiquitination cascade.

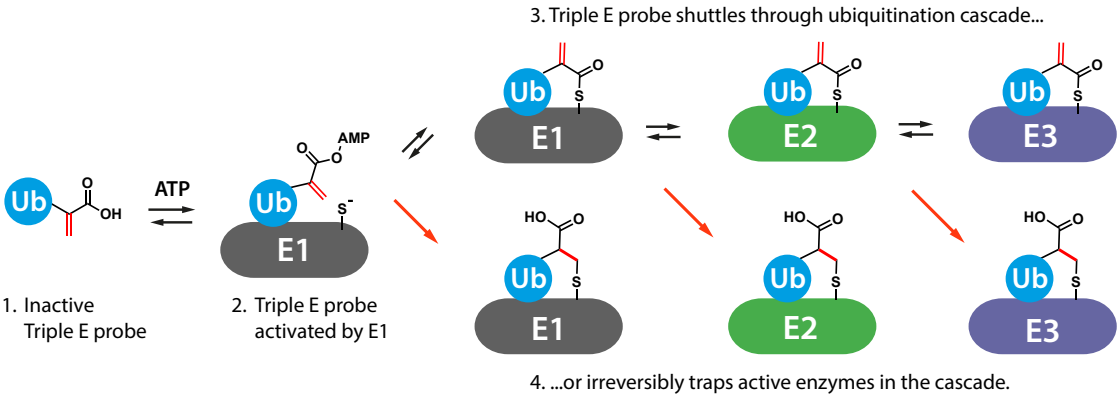

figure 1

Triple E probes contain a C-terminal dehydroalanine (Dha) group, which at physiological pH is relatively inert on its own but becomes very electrophilic upon activation by the E1 enzyme (figure 1). While the probe is processed in a native way by the E1-E2-E3 cascade, the Dha group can react with the active site cysteine residues in an irreversible way, thereby trapping the E1, E2 and E3 enzymes (figure 1).

In contrast to other probes [2], our Triple E probes represent the first tools that allow the monitoring of full E1-E2-E3 cascade activity.[1]

applications

- gel based activity protein profiling, see appendix figure 2
- cell based assays, see appendix figure 3
- structural biology, see appendix figure 4

reference

1 (a) Mulder et al., Nat. Chem. Biol. 2016, 2084 [Epub ahead of print]
(b) MPC Mulder, F. El Oualid and H. Ovaa. Adenylation enzyme inhibitors. Patent: PCT/NL2015/050596 (publication date 03 March 2016).
2 (a) Lu et al. J. Am. Chem. Soc. 2010, 132, 1748.
(b) Olsen et al. Nature, 2010, 463, 906.
(c) H. An and A.V. Statsyuk, J. Am. Chem. Soc. 2013, 135, 16948.
(d) H. An and A.V. Statsyuk Chem. Comm. 2016, 52, 2477.
(e) Kuan-Chuan Pao et al. Nat. Chem. Biol. 2016, 2045 [Epub ahead of print]
3 Page et al. Biochemistry 2012. 51, 4175.

fact sheet, May 2016

UbiQ targeting the ubiquitin system
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UbiQ Probes

CATALOGUE OF TRIPLE E PROBES

UbiQ offers a selection of Triple E probes with various substrates and *N*-Terminal tags. For custom development please contact UbiQ directly.

Tag	Substrate	Electrophile	Code	Name
Triple E probes				
–	Ub	Dha	UbiQ- 101	Ub-Dha
Biotin	Ub	Dha	UbiQ-102	Biotin-Ahx-Ub-Dha
His6	Ub	Dha	UbiQ-103	His6-Ahx-Ahx-Ub-Dha
Cy5	Ub	Dha	UbiQ-104	Cy5-Ub-Dha
5-carboxyRh110	Ub	Dha	UbiQ-131	5-carboxyRh110-Ub-Dha
–	Nedd8	Dha	UbiQ-105	Nedd8-Dha
Biotin	Nedd8	Dha	UbiQ-106	Biotin-Ahx-Nedd8-Dha
5-carboxyRh110	Nedd8	Dha	UbiQ-122	5-carboxyRh110-Nedd8-Dha

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

FAQ

Which of the E1-E2-E3 enzymes the probe will target?
The probe targets active site cysteine based E1-E2-E3 enzymes.[1]

What about RING class E3 enzymes that lack a catalytic site cysteine and thus are not directly targeted by the Triple E probe?
Highly stable E2-Ub(l)-Dha thioether adducts act as stable (competitive) inhibitors of RING type E3 ligases. This allows studies of the RING class E3 enzymes.[1]

Is the probe transferred to substrates of the E1-E2-E3 enzymes?
As far as we know the Triple E Probe is not transferred to substrates.[1a]

How specific is the probe labelling?
The ubiquitin (-like) substrate context of the probe in combination with the required activation by the E1 mediated adenylation step, makes the probe highly specific for enzymes in the ubiquitination cascade.[1]

Can the probe be used in *cell lysates* and *live cells*?
Yes, see Figure 2 and Figure 3a and 3b.[1]

Is the probe processed in a native way?
Yes, see figure 1.[1a]

Are other substrates or tags available for the probe?
Yes, other substrates will follow soon, or can already be tailor-made upon request.[1]

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APPENDIX

The Triple E probes allow the monitoring of full E1-E2-E3 cascade activity. The probes have been tested in various applications. Please see reference 1 for the details on the experimental set-up.

1 ACTIVITY-BASED PROTEIN PROFILING IN CELL EXTRACT

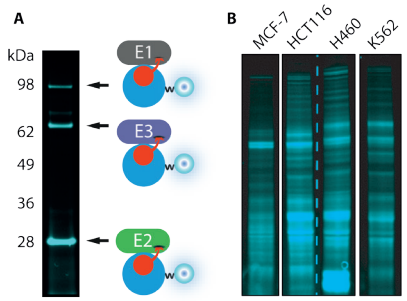

figure 2 – In-gel fluorescence imaging of
A) a mixture of E1 (Uba1), E2 (Ube2L3) and E3 (ITCH) and
B) a panel of tumor cell lines, all incubated with Cy5-*Triple E* probe and ATP.

2 CELL-BASED ASSAYS

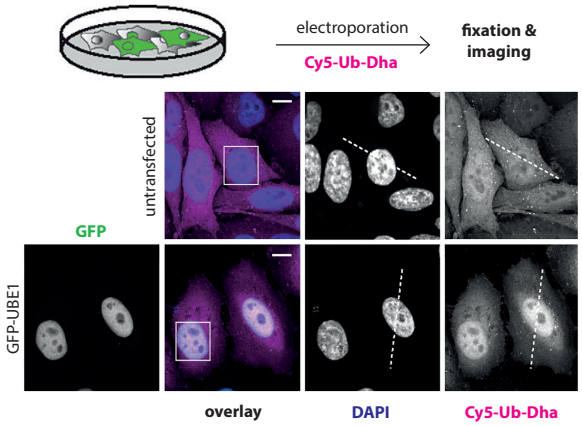

figure 3a – distribution of Cy5-Ub-Dha (magenta) in HeLa cells that electro ectopically express GFP-UBE1 (green) relative to untransfected cells.[1a]

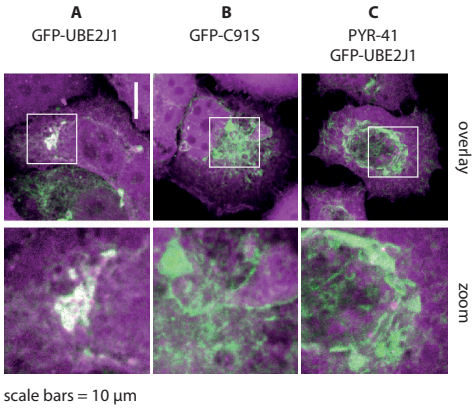

figure 3b – visualizing E2 activity in HeLa cells with (electroporated) Cy5-Ub-Dha (magenta). GFP-UbE2J1 = green, green + magenta = white. PYR-41= Uba1 inhibitor.[1a]

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APPENDIX

3 STRUCTURAL BIOLOGY

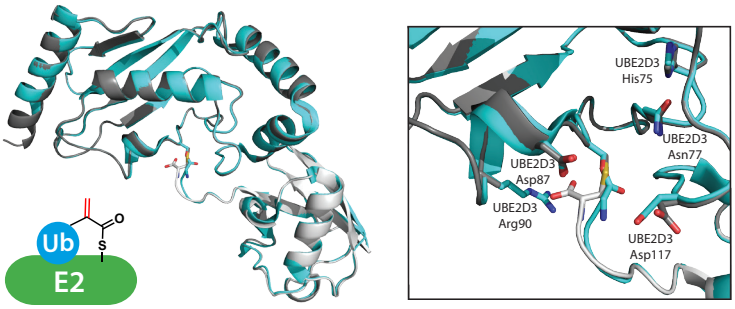

figure 4 – Structural analysis [Ub-Dha-UBE2D3] conjugate (formed via Ub E1). (1, 3)[1a]

4 PROTEOMICS

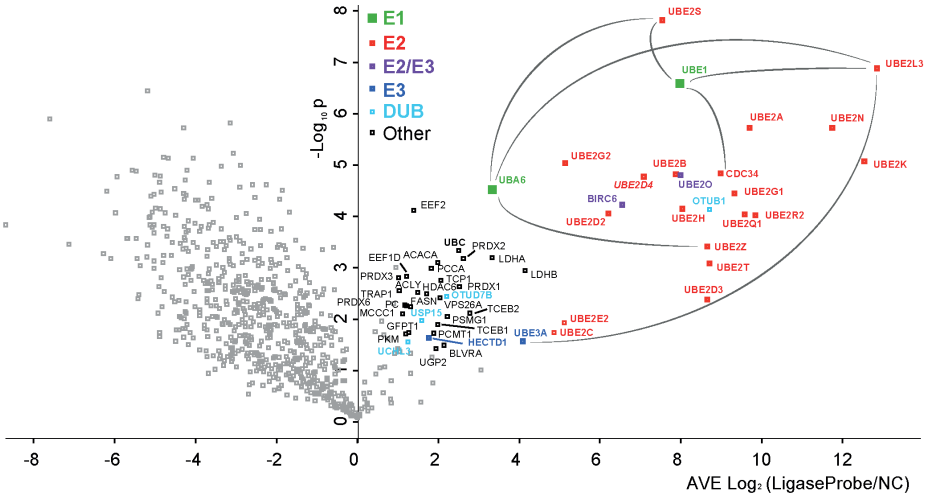

figure 5 – proteome-wide activity profiling of Ub ligation in HeLa cells.[1]

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