UbiQ

Triple E platform for drug discovery

UbiQ has developed the *Triple E* technology, which exploits a reacting group that specifically traps the E1, E2 and E3 ubiquitinating enzymes. Based on this platform UbiQ is developing small molecule inhibitors targeting the ubiquitin and ubiquitin-like E1-E2-E3 cascade.

key features of *Triple E* small molecule drugs

- target-activated prodrug
- irreversible binding
- structure-based drug design

partnering opportunity

UbiQ Therapeutics aims to expand its drug discovery efforts by teaming up with pharma partners on drug discovery programs based on our *Triple E* platform.

company

UbiQ Therapeutics, established in 2015, is a business division of UbiQ Bio, established in 2010 as a spin-out of the Netherlands Cancer Institute.
Whereas UbiQ Bio develops and commercializes ubiquitin research tools, UbiQ Therapeutics is focussing on in house and collaborative drug discovering programs in the ubiquitin field.

management team

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Boris Rodenko, PhD
VP Translational Drug Discovery

UbiQ's *Triple E* platform opens up a large pool of highly promising, yet previously poorly accessible drug targets:

E1, E2 and E3 enzymes of ubiquitin and ubiquitin-like modifiers

E1-E2-E3 ubiquitination inhibitors as novel therapeutics

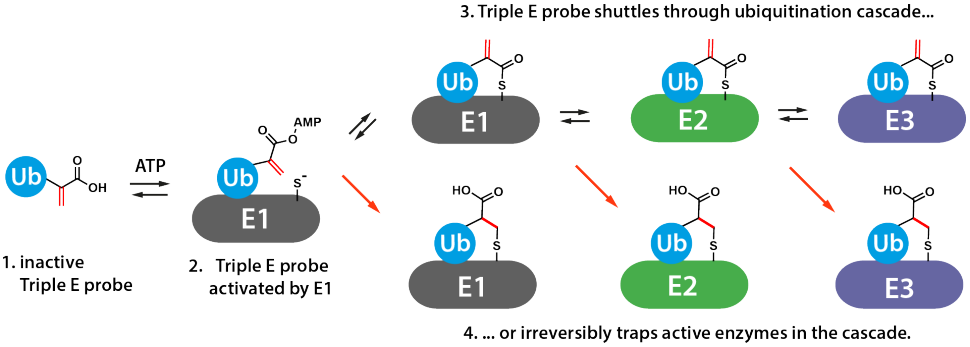
Protein ubiquitination regulates most aspects of cell life. Ubiquitin is conjugated onto a target protein via a cascade reaction involving the consecutive action of E1, E2 and E3 enzymes. The reverse reaction is executed by deubiquitinating enzymes (DUBs). Defects in these regulation mechanisms cause cancer and many other diseases. There are hundreds of enzymes responsible for ubiquitination and many of these are emerging as promising drug targets, for example in oncology. However, developing drugs which are capable of targeting these enzymes has been extremely difficult, not in the least due to the lack of suitable assay reagents and tool compounds.

the *Triple E* platform

We have developed the *Triple E* platform, which exploits a target-activated reacting group that specifically traps the E1, E2 and E3 ubiquitinating enzymes. By adding this 'hook' onto ubiquitin itself (as a research tool) or onto a small molecule (as a drug), it has now, for the first time, become possible to monitor and/or block the activity of dozens of enzymes involved in protein ubiquitination specifically. The *Triple E* platform is published in *Nature Chemical Biology* in May 2016 (http://dx.doi.org/10.1038/nchembio.2084).

mode of action

Our *Triple E* platform can best be explained by describing the action of the ubiquitin-based *Triple E* **probe** that is used as a research tool. A ubiquitinating cascade involves three types of enzymes and consists of the following steps: First, an E1 enzyme activates the C-terminus of ubiquitin by adenylation and subsequently forms an intermediate thioester. Then the active site cysteine residue of an E2 enzyme takes over ubiquitin from E1. Consequently, a transfer of ubiquitin from E2 to the active site cysteine of an E3 enzyme (HECT or RBR type) takes place. Finally, the E3 enzyme connects with its target protein and transfers ubiquitin to a lysine residue of the target protein.



Triple E probes contain a C-terminal dehydroalanine (Dha) group, which at physiological pH is relatively inert on its own (1) but becomes very electrophilic upon activation by the E1 enzyme (2). While the probe is processed in a native way by the E1-E2-E3 cascade (3), the Dha group will react with the active site cysteine residues in an irreversible way, thereby trapping the E1, E2 and E3 enzymes (4).

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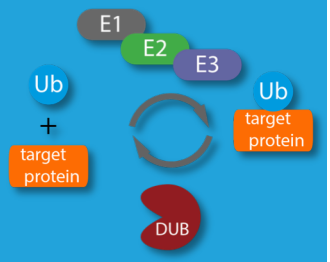
ubiquitination balance



Cellular homeostasis is maintained by the balancing action of Ub conjugating cascades featured by E1, E2 and E3 enzymes, countered by de-ubiquitinating proteases (DUBs). Both types of enzymes are promising drug targets.

market opportunity

It is expected that the ubiquitin field will prove at least as rich in drug targets as the phosphorylation system (e.g. kinases). The future market potential is promising: research into the ubiquitin field is expanding exponentially and is starting to yield validated drug targets for specific indications. A proof-of-principle of this potential is the ubiquitin-like activating enzyme inhibitor pevonedistat, which has entered phase I/II clinical trials for the treatment of multiple myeloma and non-Hodgkin's lymphoma.

proprietary position

MPC Mulder, F. El Oualid and H. Ovaa. Adenylation enzyme inhibitors. PCT/NL2015/050596 (publication date 03.03.2016)

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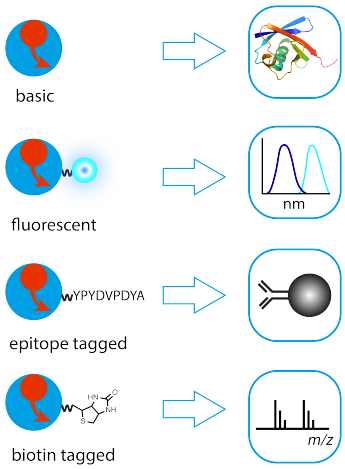
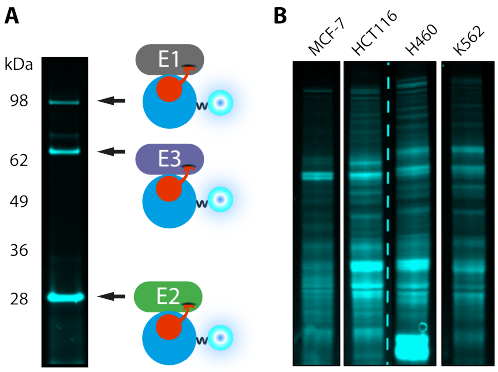
application of *Triple E* probes as research tools

Triple E probes can readily be equipped with N-terminal substitutions ranging from biotin, FLAG, HA and His-tags to dyes such as TAMRA, Cy5 and 5-carboxy-rhodamine110. As such, *Triple E* probes are invaluable for target identification and validation, e.g. in tumor cells. What is more, *Triple E* probes stabilize structures of trapped ubiquitinating enzymes and thus facilitate crystallization of these complexes. Structures of notoriously hard to crystallize ubiquitinating enzymes now come into reach. *Triple E* probes therefore expedite structure-aided design of small molecule inhibitors of these promising drug targets. Next to *Triple E* probes based on the ubiquitin sequence, we also make these variants based on ubiquitin-like modifiers, such as Nedd8 and SUMO.

***Triple E* probes for various applications**



Activity-based fluorescence imaging



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. Courtesy of Ovaa lab, Netherlands Cancer Institute, Amsterdam (NL).

application of the *Triple E* platform for drug design

UbiQ is exploiting the *Triple E* platform to design small molecule inhibitors of the E1-E2-E3 enzymes of ubiquitin and ubiquitin-like modifiers. For example, in our drug discovery program, **UbiQ-R107**, we are developing novel therapeutics to inhibit the human oncogene Myc by targeting SUMOylation. Various cancers were recently shown to be dependent on a hyper-active SUMOylation system. Intercepting this *non-oncogene addiction* blocks cell proliferation and induces apoptosis and is thereby the key rationale for UbiQ-R107.

Next to UbiQ-R107, the *Triple E* platform will open up a large pool of highly promising, yet previously poorly accessible drug targets and will guide the development of inhibitors of targets in the E1-E2-E3 conjugating cascades of ubiquitin and ubiquitin-like modifiers. The main advantages of these inhibitors are that (1) they behave as a prodrug that needs activation by its target enzymes; (2) they bind covalently to the active site of their targets; (3) their design is structure-based.

partnering opportunity

Next to extending our own pipeline, we aim to set up collaborative drug development programs together with pharma partners, based on our proprietary *Triple E* platform. Partners will gain access to our revolutionary, new technology to develop multiple E1-E2-E3 cascade inhibitors, which hold promise as first-in-class drugs in various oncology and non-oncology indications.