UbiQ
targeting the ubiquitin system

ACCELERATE DRUG DISCOVERY IN THE UBIQUITIN PROTEASOME SYSTEM

Protein ubiquitylation – the labeling of a protein with ubiquitin – directs a wide range of biological processes from protein breakdown by the proteasome, cellular localization of proteins to transcriptional activity and DNA repair. Furthermore, the successes of the proteasome proteasome inhibitor Velcade® (bortezomib), the first-to-market ubiquitin proteasome system (UPS) modulator ($3 billion USD revenue in 2014), the growth of Kyprolis®(carfilzomib) which was launched in 2012, and the recently submitted drug application of ixazomib reveal the potential of the UPS as a pharmacological target area.

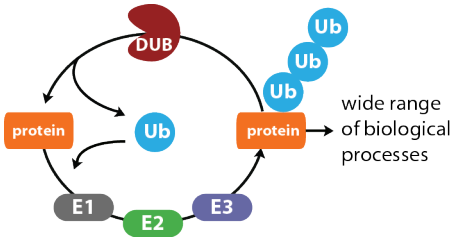

Fig 1. The ubiquitin proteasome system (UPS)

pharmacological targets in the UPS

The malfunction in UPS activity in multiple diseases has become the focus of various drug discovery efforts aimed at developing small molecule inhibitors. Despite the successes with the proteasome inhibitors, developing modulators of other enzymes in the UPS has, however, proven challenging due to a lack of research reagents and strategies. *UbiQ offers innovative reagents and an experienced team to help you to overcome these challenges.*

Ubiquitylation requires the consecutive action of E1, E2, and E3 enzymes in defined combinations to provide specificity for the protein target and to control the nature of the Ub chain topology. Deubiquitinating enzymes (DUBs) can reverse this process by cleaving the peptide or isopeptide bond between ubiquitin and its substrate protein.

Both ubiquitinating and deubiquitinating enzymes are considered promising drug targets. Human ubiquitin (-like) systems consist of about a dozen E1, several dozens of E2 enzymes, hundreds of E3 ligases and about a hundred DUBs.

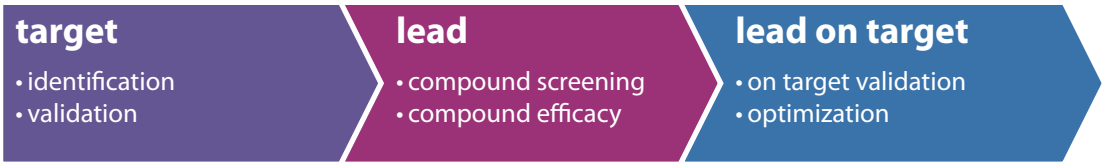

Fig 2. Three phases of drug discovery serviced by UbiQ

drug discovery

Since 2010, UbiQ's custom ubiquitin-related reagents have enabled leading pharmaceutical companies to start and make rapid progress in all three phases of early stage drug discovery. Ranging from target identification to lead discovery to lead on target validation.

The table on the next page illustrates how UbiQ's ubiquitin-related reagents can assist your early stage drug discovery in the UPS.

Science Park 408, 1098 XH Amsterdam, The Netherlands info@ubiqbio.com +31 20 303 1970 www.ubiqbio.com

UbiQ
targeting the ubiquitin system

UbiQ reagents and services help accelerate your drug discovery

target	lead	lead on target
• target identification • target validation	• compound screening	• lead on target validation • lead optimization
experiments	**experiments**	**experiments**
• target crystallization • chemical proteomics for target identification *(i.e., pull down, purification, western blotting, MS)* • phenotypic protein profiling on cell lysates for target validation	• compound library screening - library selection - finding the right screening partner - HTS activity assays, (*orthogonal, false positive minimization)*	• target inhibitor profiling: *(affinity, selectivity, and specificity profiling)* • chemical proteomics
UbiQ reagents	**UbiQ reagents**	**UbiQ reagents**
UbiQ**Probes** (page 3) activity-based probes	UbiQ**Assay** (page 4) activity assay reagents	UbiQ**Probes** (page 3) activity-based probes UbiQ**Assay** (page 4) activity assay reagents UbiQ**Chains** (page 5) all 8 natively-linked di-ubiquitin chains UbiQ**PEP** (page 5) custom ubiquitinated peptides
targets • Ub and Ubl E1, E2, E3 enzymes • DUBs and Ubl proteases • proteasome	targets • DUBs and Ubl proteases	targets • Ub and Ubl E1, E2, E3 enzymes • DUBs and Ubl proteases • proteasome
UbiQ services	**UbiQ services**	**UbiQ services**
• highly specialized custom probes, *e.g. substrate context* • activity-based phenotypic protein profiling service	• highly specialized custom assay reagents, *e.g. different dye or substrate context* • assay set-up design • screening partner selection	• highly specialized custom reagents • activity-based DUB inhibitor profiling service

---

More information on our reagents and how these can be used in early stage drug discovery on the UPS is provided in the coming pages. Please refer to our fact sheets for the technical details and available options.

fact sheet, September 2015

Science Park 408, 1098 XH Amsterdam, The Netherlands info@ubiqbio.com +31 20 303 1970 www.ubiqbio.com

UbiQ
targeting the ubiquitin system

UbiQ Probes in drug discovery

activity-based probes

UbiQ's wide selection of activity-based probes make it possible to crystallize, identify and validate dozens of enzymes as potential targets involved in protein *ubiquitination* and *de-ubiquitination*.

applications

- structural studies
- activity-based protein profiling & chemical proteomics

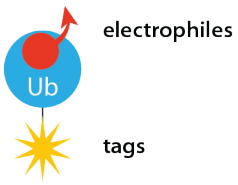

Fig 3. UbiQ-Probes, available with a range of electrophiles and tags

targets

- E1-E2-E3 enzymes (Ub and Ubl) - with UbiQ activity based *Triple E* probes
- all major DUB families (UCH, USP, and OTU) and Ubl proteases – with our activity based DUB probes
- the proteasome

experience

- in addition to various activity-based probes, UbiQ has developed more than15 custom probes for a range of customers
- numerous DUBs were successfully crystallized using UbiQ's activity-based probes, including: USP4, UCH-L5, and the viral ovarian tumor DUB (vOTU), with Ub-PA (Prg) probes. Cezanne DUB with UbiQ K11 di-ubiquitin VME probe (unpublished data)
- target validation and identification of DUBs in infectious diseases (University Glasgow / GSK)

> See UbiQ-Probes fact sheets for more information, available options, and references.

Science Park 408, 1098 XH Amsterdam, The Netherlands info@ubiqbio.com +31 20 303 1970 www.ubiqbio.com

UbiQ
targeting the ubiquitin system

UbiQ Assay in drug discovery

DUB activity assay reagents

UbiQ-Assay reagents proved effective in multiple industrial scale HTS campaigns. They are available in various flavors for a wide range of assay read outs, e.g. fluorogenic substrates for fluorescence intensity read-out, substrates for FP assays, or as *highly specialized context specific* FP reagents (our class II FP reagents). All of them targeting DUBs & Ub-like proteases.

applications

- compound library screening
- activity assays

targets

- DUBs and Ubl proteases

Fig 4. UbiQ-Assay class II reagents harbour a substrate-based peptide, connected via a native isopeptide bond.

experience

- substrate supply for an undisclosed number of large library screens by our industrial partners
- development of >10 *highly specialized context specific* custom assay reagents
- demonstrated performance at independent European screening centers – Pivot Park Screening Center and European ScreeningPort (see Table 2)
  - five DUB screening campaigns on over 1.7 million small molecules

Table 2. UbiQ's screening reagents used in HTS campaigns*

screen#	primary screen + dose-response	orthogonal assay for hit validation	small-molecule library size	Z'-value	validated hits	hits selected for dose-response
DUB-001	UbiQ-012 (Ub FP)	UbiQ-002 (Ub-Rh110)	225K	0.7 - 0.8	293 (0.14%)	99 (0.05%)
			310K	0.7 - 0.8	664 (0.21%)	182 (0.06%)
DUB-002	UbiQ-012 (Ub FP)	UbiQ-002 (Ub-Rh110)	310K	0.7 - 0.8	516 (0.17%)	152 (0.05%)
DUB-003	Ub-like FP reagent	Ub-like (AMC)	310K	0.7 - 0.8	1009 (0.33%)	170 (0.05%)
DUB-004	Ub-like FP	Ub-like (AMC)	310K	0.7 - 0.8	*ongoing*	*ongoing*
DUB-005	Ub FP class II reagent	UbiQ-002 (Ub-Rh110)	310K	0.75 - 0.8	*ongoing*	*ongoing*

*Completed and ongoing HTS programs in which UbiQ's FP assay reagents were used for the primary screen (single point at 10 µM), and dose-response experiments of validated hits. All assays showed excellent Z' values (>0.7). After confirmation of the hits with the FP substrate, an orthogonal Ub-Rh110Gly (UbiQ-002) or Ub-like AMC assay was used for validation; the number of hits identified at this stage varied from 0.33–0.14% of the total library. A subset of compounds (in general 0.05% of the total library) is then selected for IC_{50} determination using the FP assay reagent (10 concentration steps from 0.15 nM–5 µM).

> See UbiQ-Assay fact sheets for more information, available options and references.

Science Park 408, 1098 XH Amsterdam, The Netherlands info@ubiqbio.com +31 20 303 1970 www.ubiqbio.com

UbiQ
targeting the ubiquitin system

UbiQ^Chains in drug discovery

all 8 natively-linked di-ubiquitin chains

UbiQ is the original and only manufacturer of all 8 di-ubiquitin chain linkages, each of which have been identified in cells. Therefore, access to all 8 linkages is key to further investigating their biological roles.

applications

- studying linkage specificity of proteases
- mechanism of binding and recognition of proteins that contain ubiquitin-associated domains or ubiquitin-interacting motifs (UIMs)
- di-ubiquitin probes and assay reagents are also available

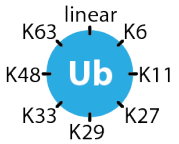

Fig 5. UbiQ-Chains available on all lysine linkages and linear

targets

- DUBs and Ubl proteases

experience

- several linkage-specific DUBs have been identified, including: Cezanne (K11), TRABID (K29 and K33), human AMSH-LP (Lys 63), and USPs showing a specific di-ubiquitin linkage preference.

UbiQ^PEP in drug discovery

custom ubiquitinated peptides

UbiQ provides ubiquitinated peptides with a range of custom modifications to meet your research needs. Peptides up to 20 amino acids long are site-selectively ubiquitinated via a native isopeptide link using our proprietary Ub ligation technology.

applications

- epitope mapping (e.g. with DUBs) and immunization
- peptide pull-down experiments (proteomics)
- ligand for X-ray analysis of proteins

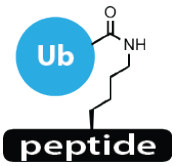

Fig 6. UbiQ-PEP, ubiquitinated peptides with a native isopeptide bond

targets

- Ub and Ubl E1, E2, E3 enzymes, DUBs and Ubl proteases, and proteasome

experience

- UbiQ has successfully developed site-specific ubiquitin antibodies (unpublished data)
- peptide pull-down assays with custom H2B & PTEN ubiquitinated peptides [Kessler, R. et al. *Nat. Commun.*, 2015, *6*, 7049.]

> See UbiQ-Chain and *UbiQ-PEP fact sheets for more information, available options, and references.*

fact sheet, September 2015

Science Park 408, 1098 XH Amsterdam, The Netherlands info@ubiqbio.com +31 20 303 1970 www.ubiqbio.com