



Group leader Huib Ovaa

Huib Ovaa PhD Group leader
Boris Rodenko PhD Senior post-doc
Alessia Amore PhD Post-doc
Farid El Oualid PhD Post-doc
Remco Merckx PhD Post-doc
Sander van Kasteren PhD Post-doc
Paul Geurink PhD Post-doc
Celia Berkers PhD Post-doc
Harald Albers MSc PhD student
Annemieke de Jong MSc PhD student
Reggy Ekkebus MSc PhD student
Rieuwert Hoppes MSc PhD student
Henk Hilkmann Ing Technical staff
Dris El Atmioui Ing Research assistant
Kim Wals Ing Research assistant
Yves Leestemaker MSc Research assistant
Dharjath Hameed MSc Research assistant

Publications

Albers HM, Dong A, van Meeteren LA, Egan DA, Sunkara M, van Tilburg EW, et al. *Boronic acid-based inhibitor of autotaxin reveals rapid turnover of LPA in the circulation. Proc Natl Acad Sci U S A.* 2010;107:7257-62

Albers HM, van Meeteren LA, Egan DA, van Tilburg EW, Moolenaar WH, Ovaa H. *Discovery and optimization of boronic acid based inhibitors of autotaxin. J Med Chem.* 2010;53:4958-67

Berkers CR, de Jong A, Ovaa H, Rodenko B. *Transpeptidation and reverse proteolysis and their consequences for immunity. Int J Biochem Cell Biol.* 2009;41:66-71

Berkers CR, Ovaa H. *Drug discovery and assay development in the ubiquitin-proteasome system. Biochem Soc Trans.* 2010;38:14-20

Cavalli S, Houben AJ, Albers HM, van Tilburg EW, de Ru A, Aoki J, et al. *Development of an activity-based probe for autotaxin. ChemBioChem.* 2010;11:2311-7

Hoppes R, Ekkebus R, Schumacher TN, Ovaa H. *Technologies for MHC class I immunoproteomics. J Proteomics.* 2010;73:1945-53

CHEMISTRY AND BIOLOGY OF UBIQUITIN-MEDIATED PROTEOLYSIS AND ANTIGEN PRESENTATION

The proteasome is a multi-catalytic proteolytic machine that is abundant and responsible for the turnover of many critical regulatory proteins including tumor suppressor proteins and cell cycle regulators. The destructive force of the proteasome as an important determinant of protein half-life is regulated by ubiquitination. Substrates are tagged with multiple ubiquitin (Ub) molecules for destruction by the proteasome. Ubiquitin is a 76 amino acid protein that can be conjugated onto substrates to guide protein destruction. The majority of proteins are targeted for proteasomal proteolysis by Ub polymers. Despite a wealth of literature on ubiquitination of proteasome substrates, little is known about the degradation process at a more detailed molecular level; ubiquitination status and protein stability currently cannot be predicted. It is clear however, that a ubiquitin code exists and that protein turnover by the proteasome is a tightly regulated and complex process that includes not only the complexity of the proteasome but also Ub polymer formation and remodeling and Ub recycling. Because of this complexity, tools that allow detailed studies of the effects of ubiquitination status on protein turnover are urgently needed.

Our lab aims to develop tools to profile cellular enzymatic activities associated with post-translational modification of proteins with ubiquitin and we study proteasome activity, antigen production and antigen presentation by designing tools that interfere with individual components of these systems. We search for inhibitors of enzymatic activities and ligands of receptors both by high throughput screening of small molecule compound collections using *in vitro* biochemical screens and cell-based assays and by rational chemical design followed by chemical optimization and all biochemistry further required. Ligands and inhibitors form the basis for the development of research probes that we use to achieve our goals.

Research is centered around one central theme: **chemistry and biology of ubiquitin-mediated proteolysis and MHC class I antigen presentation**, and divided into three main topics:

- (1) ubiquitin chain chemistry, biochemistry and proteomics
- (2) proteasome inhibition and activation
- (3) MHC class I antigen presentation

Chemical Synthesis of Ubiquitin conjugates By the concerted action of E1, E2 and E3 enzymes, Ub is linked to target proteins via an isopeptide bond between the Ub C-terminal carboxylate and the ϵ -amine of a lysine residue or N-terminus of the target protein. The ability to generate well-defined Ub conjugates biochemically is limited by the requirement of prior identification and production of specific E2/E3 enzymes. In addition, the generation of Ub mutants by biochemical methods is limited to the natural amino acid repertoire. To have full control over Ub conjugation and structure, we have recently developed chemical approaches that allow us to synthesize virtually any well-defined Ub conjugate and mutant (figure 2). Using

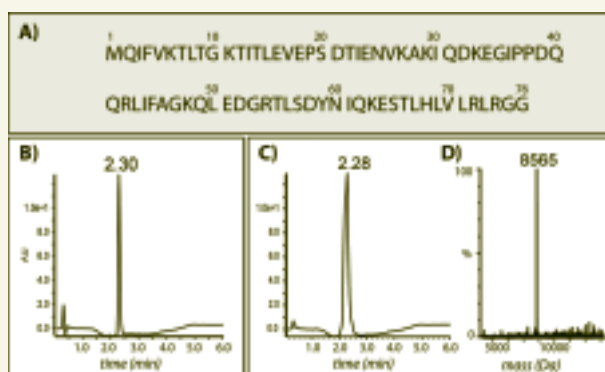


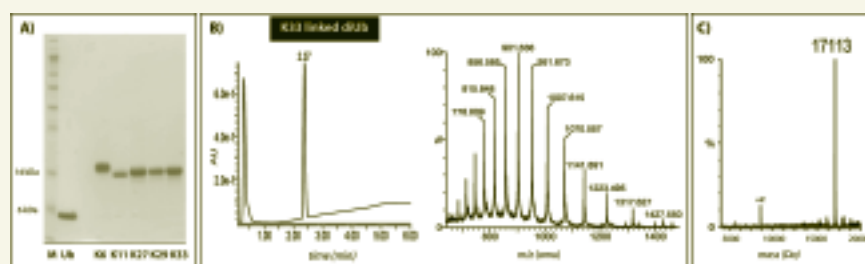
Figure 2:
 A) Amino acid sequence of Ubiquitin. B) LC profile of a commercial Ubiquitin sample and C) our crude synthetic Ubiquitin. D) Mass spectrometric analysis of crude synthetic Ub, calc 8565 Da, found 8565 Da (deconvoluted spectrum right).

these chemistries, we prepared fluorogenic Ub conjugates and showed that these are fully functional (figure 3). In another synthetic effort we focused on the ability of Ub to be self-conjugated onto any of its 7 lysine residues (*i.e.* K6, K11, K27, K29, K33, K48 and K63). Although all linkages have been identified in cells, only K11, K48- and K63-linkages have been studied so far as these are the only linkages that can currently be obtained biochemically. To overcome this limitation, we have developed a very effective synthetic approach towards all seven isopeptide linked diUb conjugates. Figure 4 shows analysis of the synthesized K6, K11, K27, K29 and K33 linked diUb conjugates. Overall, routine strategies for the chemical construction of Ub mutants, Ub chains or specific C-terminal modifications, now bring virtually any Ub derivative within practical reach.

Chemical reporters of UPS activity Pharmacological interference with UPS-mediated protein degradation holds much promise. However, the only example of pharmacological modulation of the UPS approved for use in the clinic so far is the proteasome inhibitor bortezomib and tools to study proteasome action are in demand. A chemical approach using irreversible covalent inhibitors equipped with reporter groups offers several advantages over traditional approaches, including their applicability to any cell line or tissue. We recently developed such probes which have been shown to provide information that correlates directly with the functional state of enzyme active sites: active forms only and not latent or (auto)inhibited activities are reported. Using fluorescent proteasome activity reporters we have analyzed proteasome activity in mice and we were able to monitor pharmacological inhibition *in vivo* and to visualize active proteasomes in cells both by confocal microscopy and flow cytometry.

Conditional MHC class I ligands for epitope mapping MHC-bound peptides are essential for the stability of the MHC class I complex. Hence, standard strategies for the production of recombinant MHC complexes are based on *in vitro* refolding reactions with specific peptides and this severely limits the ability to produce large collections of peptide-MHC complexes. To address this issue, we developed in collaboration with the Schumacher lab conditional MHC ligands that can be cleaved in the MHC bound state under conditions that do not affect the integrity of the MHC structure. MHC class I molecules can efficiently be produced with these conditionally cleavable peptide ligands. These UV-labile ligands have been shown to disintegrate in the MHC-bound state upon exposure to UV light under mild conditions (neutral pH, 4-37°C). Importantly, when UV-mediated cleavage is performed in the presence of another MHC binding peptide, an efficient ligand exchange reaction results in a class I molecule complexed with the epitope of choice. Ligands that disintegrate on command have now been identified for various different human MHC alleles indicating that it is straightforward to identify conditional ligands for other MHC class I alleles. Furthermore, this MHC exchange technology has been adapted to high-throughput applications in automated ELISA and fluorescence polarization assays.

Improvements and development of new technologies to profile enzymatic UPS-related activities and MHC loading will allow new approaches to understanding the ubiquitin proteasome system and antigen presentation, unraveling novel phenomena. Despite major achievements in chemical probe development and in chemical investigations into the ubiquitin proteasome system and MHC class I antigen presentation, many challenges remain.



Publications (continued)

Shanmugham A, Fish A, Luna-Vargas MP, Faesen AC, El Oualid F, Sixma TK, et al. *Nonhydrolyzable ubiquitin-isopeptide isosteres as deubiquitinating enzyme probes.* *J Am Chem Soc.* 2010;132:8834-5

Verzijlbergen KF, Menendez-Benito V, van Welsom T, van Deventer SJ, Lindstrom DL, Ovaa H, et al. *Recombination-induced tag exchange to track old and new proteins.* *Proc Natl Acad Sci U S A* 2010;107: 64-8

Ovaa H, Kuijl C, Neeffjes, J. *Recent and new targets for small molecule anti-cancer agents.* *Drug Discovery Today: Technologies.* e3-e11

Frederiks F, Stulemeijer IJE, Ovaa H, van Leeuwen F. *A modified epigenetics tool box to study histone modifications on the nucleosome core.* *ChemBiochem.* 2010 2010;12:308-313

El Oualid F, Merckx R, Ekkebus R, Hameed DS, Smith J, de Jong A, et al. *Chemical Synthesis of Ubiquitin, Ubiquitin-Based Probes, and Diubiquitin.* *Angew Chem Int Ed Engl.* 2010 2010;49:10149-10153

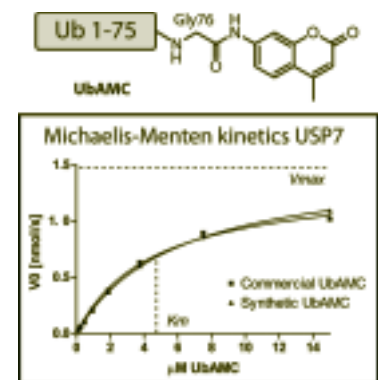


Figure 3: Turnover of commercial and synthetic UbAMC by the deubiquitinating enzyme USP7 shows identical Michaelis-Menten kinetics.

Figure 4: A) SDS-PAGE analysis of synthetically prepared K6, K11, K27, K29 and K33 linked diUb conjugates. B) LC-MS analysis of purified K33 linked diUb. C) Deconvoluted mass spectrum of K33 linked diUb (calc 17112 Da, found 17113 Da)