Modular Access to Structurally Defined Ubiquitin Chains

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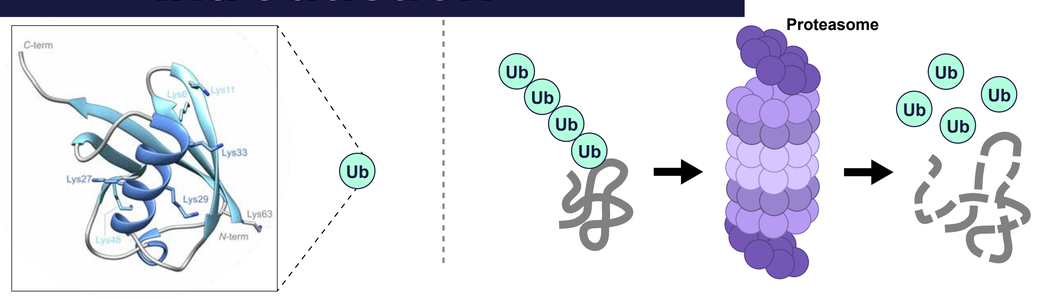
TARGETED PROTEIN DEGRADATION

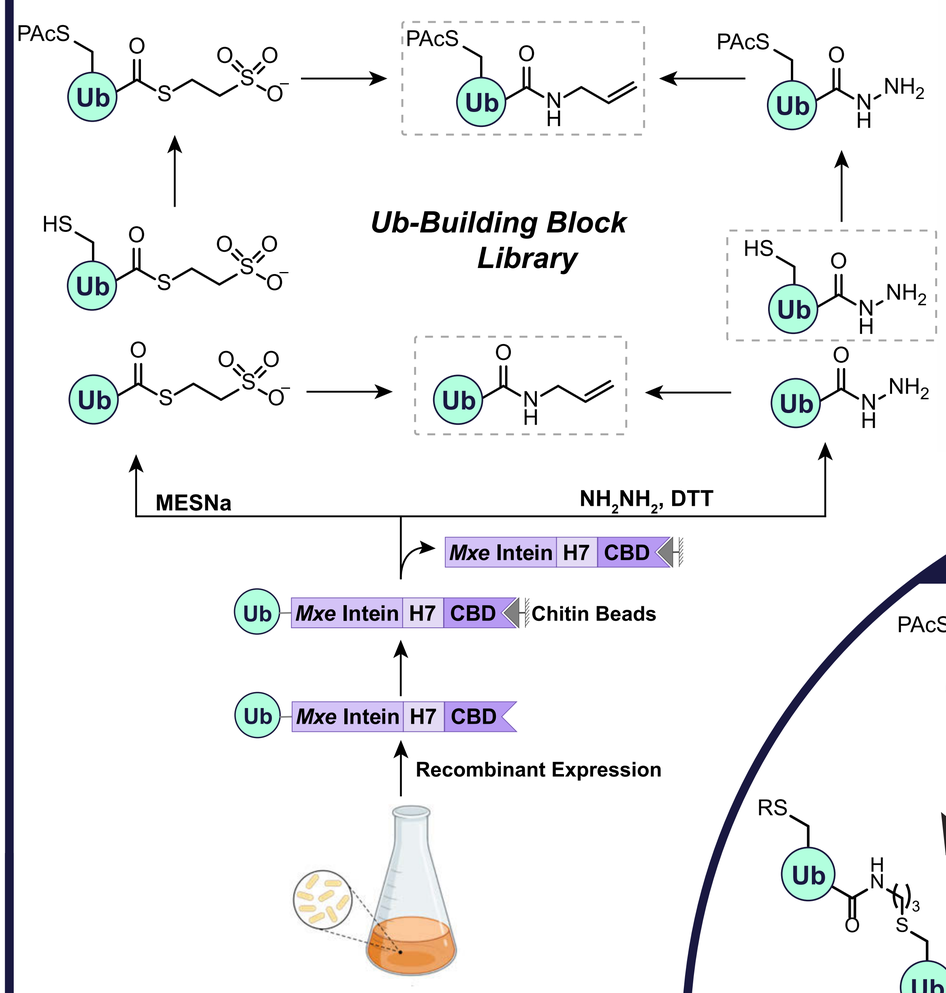
Introduction



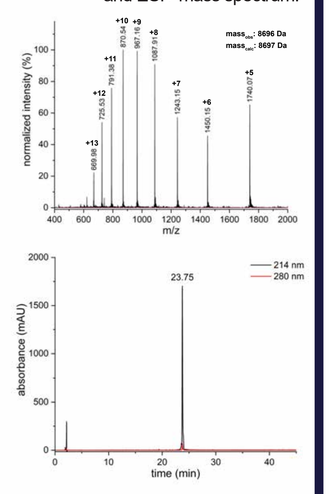
Exact molecular composition of Ubiquitin (Ub) tags is critical for executed function like degradation signalling for the ubiquitin-proteasome system (UPS) or autophagy-lysosomal pathway.
High complexity by 8 different linkage types within Ub, chain length variability, PTM of Ub means challenging assembly of defined Ub chains and ubiquitylated proteins.
Methods and strategies to generate ubiquitin chains: enzymatic approaches,[2] genetic code expansion, (semi)-synthesis.[3, 4]
Fully defined Ub chains are unique tools in deciphering complex degradation signals at a high molecular resolution.

Building Blocks

- Monomeric Ub building blocks for high flexibility in modular chain assembly
- Efficient generation of Ub-Intein fusions by recombinant expression up to 60 mg/L
- Activated Ubiquitin for universal C-terminal modification[5]/elongation



Analysis of Ub[K48C(PAc)]-AA. HPLC chromatogram and ESI+ mass spectrum.



Concept

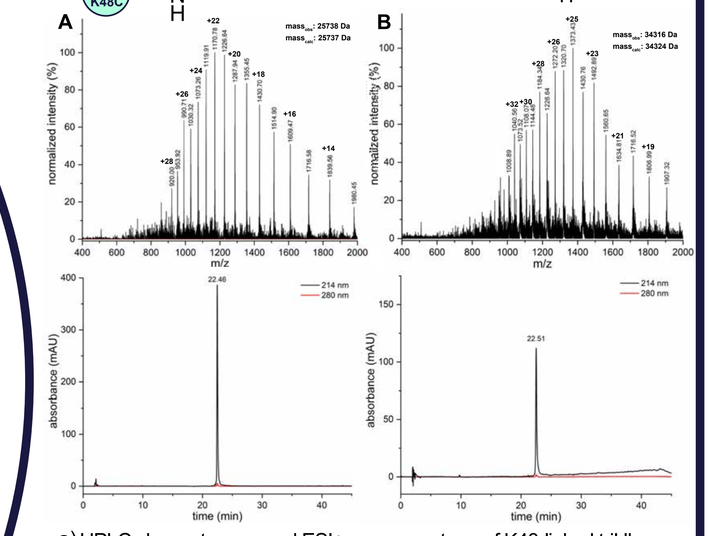
- Photoinitiated thiol-ene click (TEC) reaction for chain assembly and transfer to POI.
- Rapid, mild, chemoselective connection chemistry.
- Modular approach gives access to homotypic and heterotypic structures.
- Protection group and masking strategy for flexible bidirectional elongation.

Chain Assembly

- Iterative TEC + activation or deprotection towards Ub oligomers
- 6 mM Ub-AA + 7.5 mM UbKxxC-NHNH2 + 4 mM LAP + 1.5 mM TCEP < 1 min irradiation, up to 40% isolated diubiquitin
- Probed for UbK63C, UbK29C, UbK48C, UbK27C, UbK11C

RP-HPLC of TEC between UbAA and Ub(K48C)$NHNH_2$ before irradiation and after 45 s irradiation.

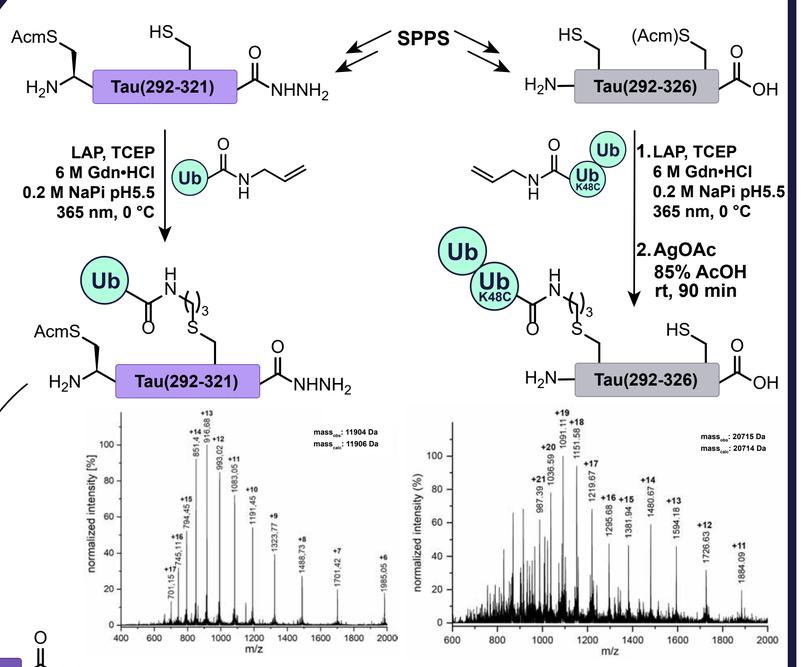
ESI+ mass spectrum of K48-linked diUb-AA.



a) HPLC chromatogram and ESI+ mass spectrum of K48-linked triUb.
b) HPLC chromatogram and ESI+ mass spectrum of K48-linked tetraUb.

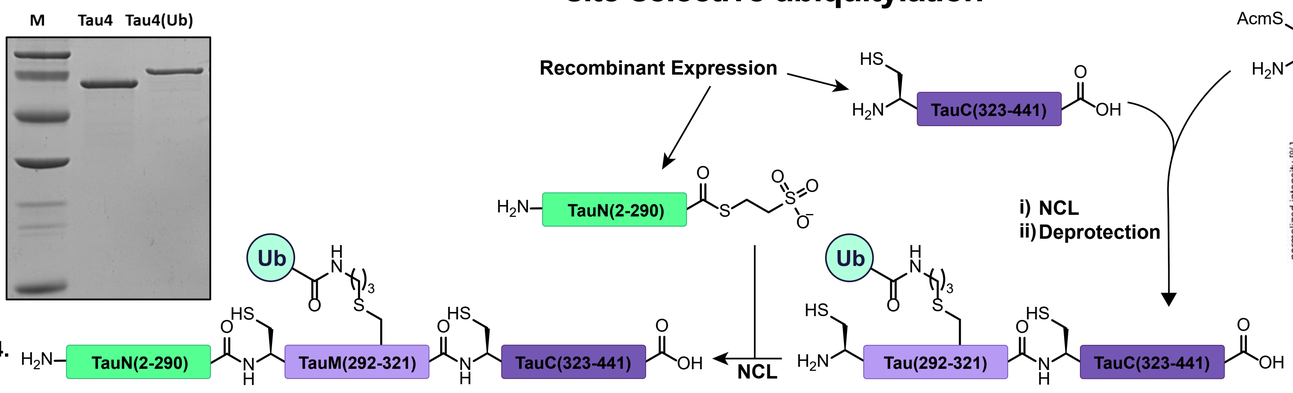
Protein Modification

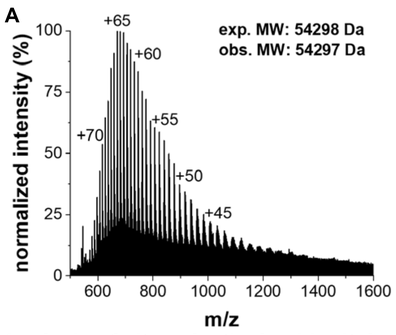
1. Ubiquitylation of peptides via TEC



Analysis of site-selectively ubiquitinated peptides.
(A) ESI+ mass spectrum of monoubiquitinated TauM(292-321);
(B) ESI+ mass spectrum of diUb(K48)-Tau(292-326).

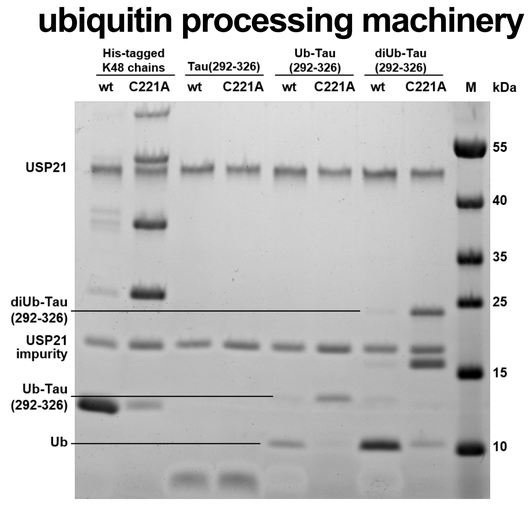
2. Construction of protein with site-selective ubiquitylation





Analysis of site-selectively ubiquitylnated Tau4. (A) ESI+ mass spectrum; (B) SDS-PAGE of native Tau4 and Tau4(Ub), coomassie stain.

3. Artificial linkage is recognized by ubiquitin processing machinery



DUB-assay. SDS-PAGE after incubation of Ub-Tau(292-326) and diUb-Tau(292-326) with USP21 @37°C for 60 min. Substrate-DUB ratio 3:2. C221A mutant as inactive control. Coomassie stain.

References

[1] D. Komander, M. Rape, Annu Rev Biochem **2012**, 81, 203-229.
[2] K. Martinez-Fonts, A. Matouschek, Biochemistry **2016**, 55, 1898-1908.
[3] Y. Huppelschoten, G. J. Van der Heden van Noort, Semin. Cell Dev. Biol. **2022**, 132, 74-85.
[4] E. M. Valkevich, R. G. Guenette, N. A. Sanchez, Y. Chen, Y. Ge, E. Strieter, J. Am. Chem. Soc. **2012**, 134, 6916-6919.
[5] K. R. Love, R. K. Pandya, E. Spooner, H. L. Ploegh, ACS Chem. Biol. **2009**, 4, 275-287.

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