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Probing the interplay between Ubiquitination and Adenosine-Di-Phosphate ribosylation

ADP-ribosylation of Ub

The already difficult Ubiquitin code has recently been exposed to be even more complex as Ub can be post-translationally modified itself. Attachment of Adenosine-Di-Phosphate ribose (ADPr) on multiple distinct amino acids positions, such as Arg42, Thr66 or Gly76 in Ub leads to the formation of a variety of isotypes of Ub^{ADPr}. These modifications are tightly regulated by transferase and hydrolase enzymes that are involved in bacterial infections (by *Legionella pneumophila* and *Chromobacterium violaceum*) and mammalian DNA damage responses although the details of these processes are poorly understood. Unveiling this new layer of ubiquitin regulation and the molecular mechanisms and cell biological consequences of these distinct Ub^{ADPr}-isotypes will benefit from well-defined tools. We here report the chemical synthesis and application of Ub^{ADPr} substrates and activity-based probes.

Development of reagents and probes

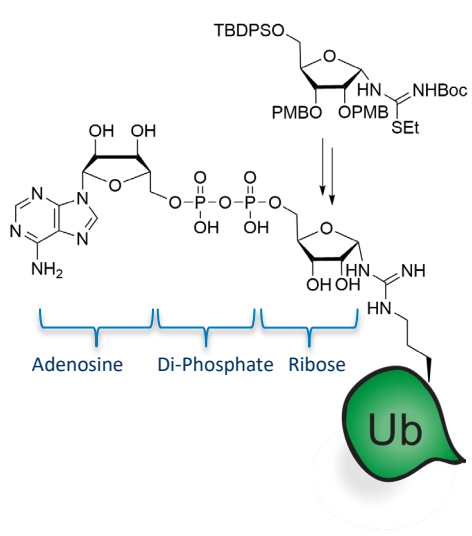
Advantages of chemical synthesis:
- ADPr introduction possible on all amino acid positions
- Attachment of non-native amino acids, affinity-tags, fluorophores or warheads

Application potential:
- Biophysical studies on enzymes (kinetic parameters/ affinity/ selectivity preferences)
- Activity-based protein profiling (discovery of interactors, undisclosed enzymes)
- Structure determination (X-ray crystallography)

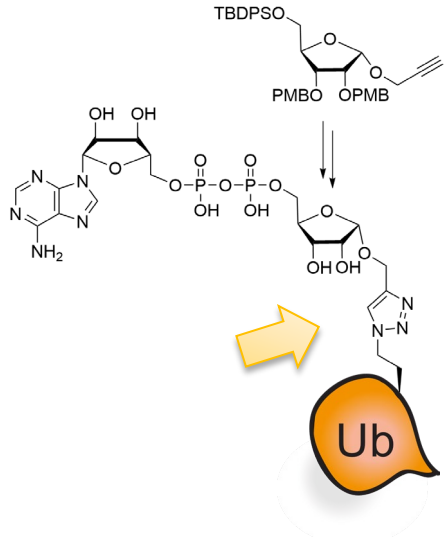
Ub^{ADPr} - toolbox

What can we do with new chemical methodologies?

native Arginine link[1]



partially stabilised[2]



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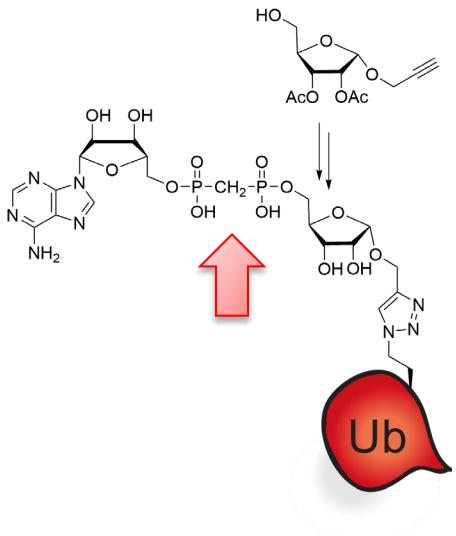
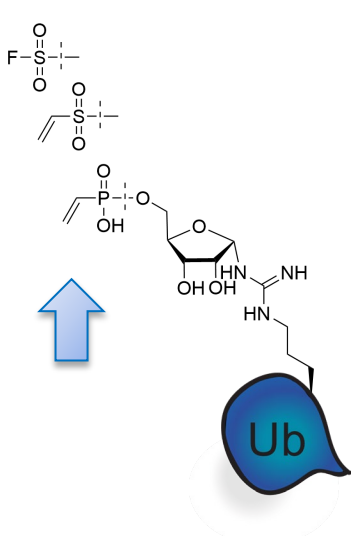
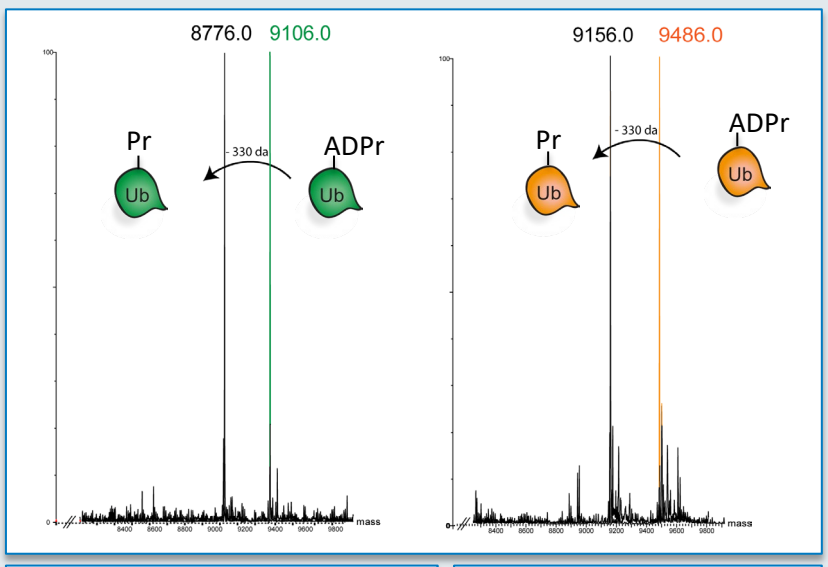
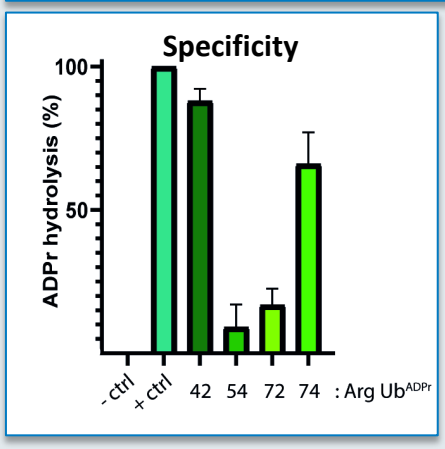


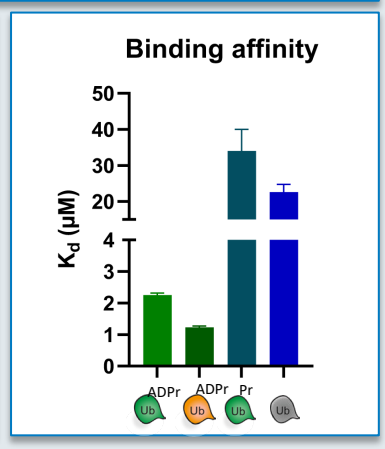
activity-based probes



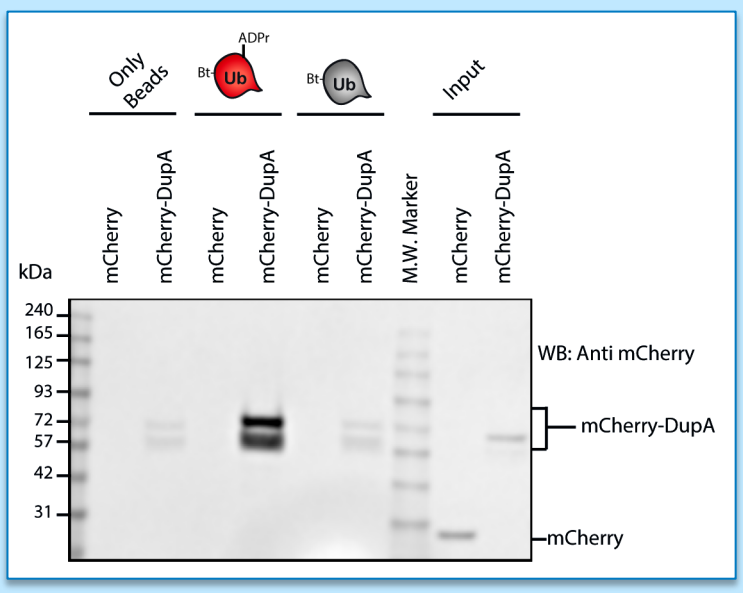
Profiling Legionella DupA activity



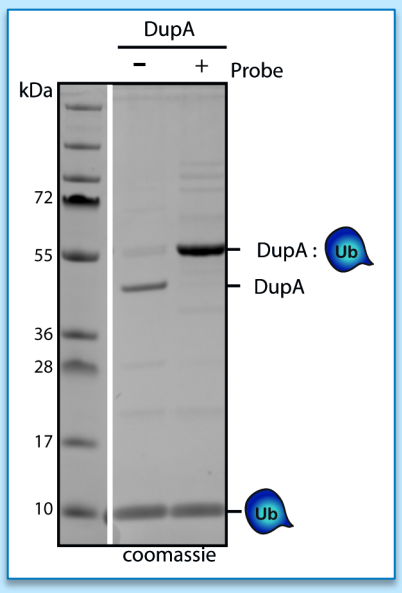




Pulldown & labelling of DupA



non-covalent interaction



covalent adduct

Outlook

The newly developed chemical methodologies towards Ub^{ADPr}-substrates showcase the power of protein synthesis. This toolbox of Ub^{ADPr}-substrates and probes and proof-of-principle studies on Legionella effector enzyme DupA provides a platform to further interrogate the cell biology affected by all Ub^{ADPr} isotypes and opens the way to finding out if this modification is more prevalent then is known to date.

References: **[1]**] J. Voorneveld, M. S. Kloet, R. Q. Kim, A. Moutsiopoulou, M. Misra, I. Dikic, , D. V. Filippov, G. J. van der Heden van Noort, *submitted for publication* **[2]** Q. Liu, H. A. V. Kistemaker, S. Bhogaraju, I. Dikic, H. S. Overkleeft, G. A. van der Marel, H. Ovaa, G. J. van der Heden van Noort, D. V. Filippov, *Angewandte Chemie-International Edition* **2018**, *57*, 1659-1672 **[3]** R. Q. Kim, M. Misra, A. Gonzalez, I. Tomaskovic, D. Shin, H. Schindelin, D. V. Filippov, H. Ovaa, I. Dikic, G. J. van der Heden van Noort, *Chemistry,* **2021**, *27*, 2506-2512

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