PHOTOACTIVE CHITOOLIGOSACCHARIDE (COS) PROBES TO IDENTIFY AND ISOLATE LYSOSOMAL OLIGOSACCHARIDE TRANSPORT (LOST)

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Small polymannose-type oligosaccharides (fOS) that are generated during protein *N*-glycosylation are transported from the cytosol into lysosomes to be degraded [1]. Presently, lysosomal oligosaccharide transport (LOST) remains to be identified at the molecular level. In terms of its activity, *in vitro* studies using rat liver lysosomes revealed that radioactive [^{3}H]Man$_5$-GlcNAc ([^{3}H]M$_5$) import is blocked by GlcNAc but not by mannose. Moreover, [^{3}H]M$_5$ transport is blocked efficiently by chitooligosaccharides containing 2 to 4 residues of β1-4 linked GlcNAc (COS2-4) with intact reducing end [2]. These data indicate that LOST may have a wider tolerance for the substrate than initially imagined and suggest that it could potentially have diverse transport functions. However, characterising LOST faces important obstacles as the protein, or protein complex, involved has not been identified. To have a better understanding of this process, we are developing chemical tools to characterize and identify LOST proteins.

Useful probes to study LOST were obtained after enzymatic de-*N*-acetylation of the non-reducing end GlcNAc residue of COS2-4. The resulting free amine allowed incorporation of a fluorophore, rhodamine B, and the resulting fluorescent probe was shown to be transported into lysosomes by LOST or a similar process [3,4]. Following this strategy, we synthesized a new generation of COS-derivatized probes containing a photoreactive moiety, a benzophenone, as well as a precursor for a reporter tag, norbornene, allowing the incorporation of tetrazine derivatized compounds, including biotin or fluorophores, after the photo-crosslinking with LOST [Figure 1] . We aim to track fluorescent probes in semi-intact cells using confocal microscopy and to isolate covalently biotinylated proteins for further study by proteomics.

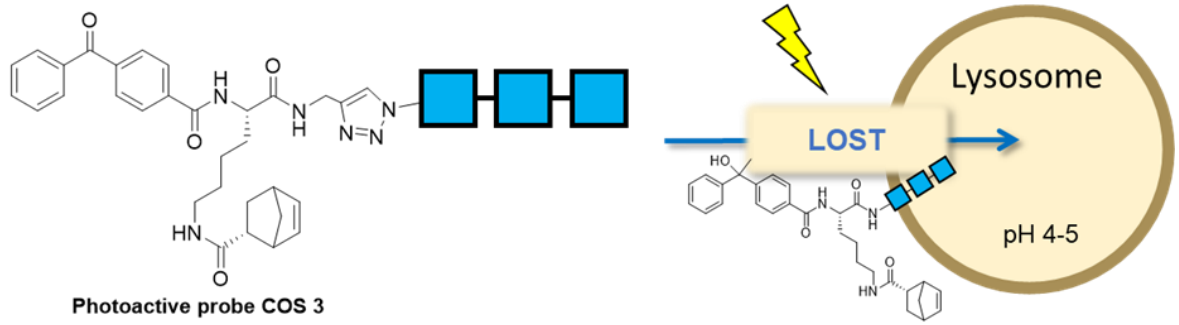

Figure 1. *Photo-cross linking of the COS-derivatized probes with LOST*

The synthesis of the probes will be presented as well as their use in rat liver lysosomes or lysosomes membranes for LOST labelling.

References:
1. A. Saint-Pol, C. Bauvy, et al., *J. Cell Biol.*, 1997, **136**, 45–59.
2. A. Saint-Pol, P. Codogno, et al., *J. Biol. Chem.*, 1999, **274**, 13547–13555.
3. R. Chambon, S. Pradeau, et al., *Carbohydr. Res.*, 2017, **442**, 25–30.
4. Y. Bouzidi, M. Bosco, et al., *Glycobiology*, 2024, **34**, cwad099.