

SYNTHESIS AND SCREENING OF RIBITOL-5-PHOSPHATE METABOLIC LABELLING TOOLS TO STUDY MUSCULAR DYSTROPHIES

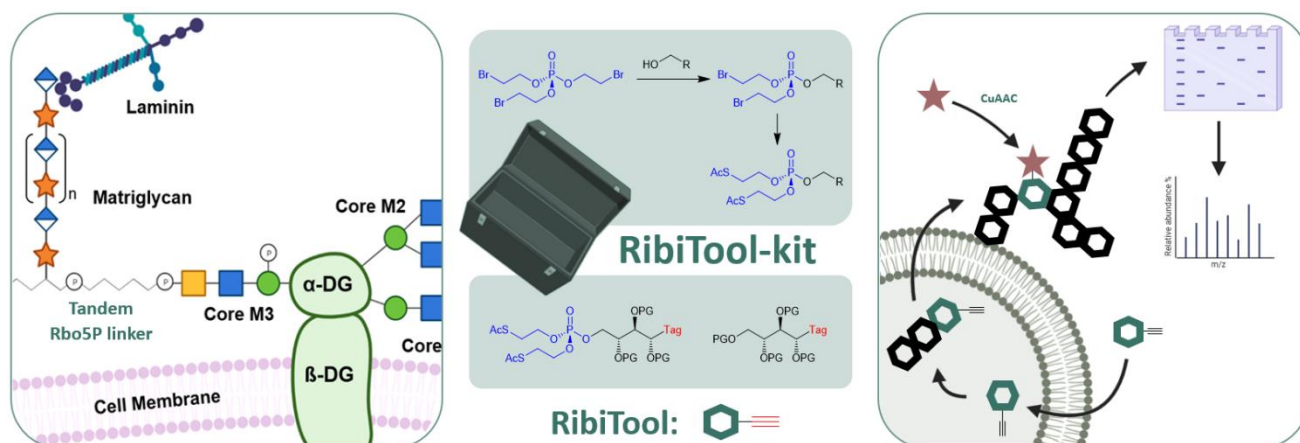
Lloyd D. Murphy, Lianne I. Willems

York Structural Biology Laboratory, Department of Chemistry, University of York,
Heslington, YO10 5DD, UK
lloyd.murphy@york.ac.uk

α -Dystroglycan (α -DG) is a highly glycosylated cell surface protein essential for binding interactions with laminin within the extracellular matrix, playing a critical role in skeletal muscle, peripheral nerve, and brain tissues. A key glycan, core M3, contains a unique tandem ribitol-5-phosphate (Rbo5P) linker, first identified in mammalian cells in 2016 [1,2]. Disruptions in Rbo5P biosynthesis are linked to congenital muscular dystrophies, highlighting the need for new tools to study its presence and function.

This work describes the development of novel alkyne-tagged ribitol and Rbo5P metabolic labeling tools, synthesised using a newly established methodology for installing cell-labile protected phosphates without the use of hazardous phosphoramidite intermediates [3]. The scope of this approach was explored, enabling the efficient synthesis of multiple probes. These metabolic tools were applied to mammalian cells, and their biological activity was assessed via bioorthogonal reactions with azide-containing reporter groups, followed by in-gel fluorescence and mass spectrometry analysis.

This study presents the first chemical tool capable of fluorescently labeling overexpressed α -DG, allowing for direct probing of mammalian cells. Expanding the scope of this methodology will facilitate broader applications in glycoprotein research and metabolic labeling strategies.



References:

1. I. Gerin, B. Ury, I. Breloy, C. Bouchet-Seraphin, J. Bolsee, M. Halbout, J. Graff, D. Vertommen, G. G. Muccioli, N. Seta, J-M. Cuisset, I. Dabaj, S. Quijano-Roy, A. Grahn, E. Van Schaftingen and G. T. Bommer, *Nat. Comm.*, 2016, **7**, 11534.
2. J. L. Praissman, T. Willer, M. O. Sheikh, A. Toi, D. Chitayat, Y-Y. Lin, H. Lee, S. H. Stalnakker, S. Wang, P. K. Prabhakar, S. F. Nelson, D. L. Stemple, S. A. Moore, K. W. Moreman, K. P. Campbell and L. Wells, *Elife*, 2016, **5**, e14473.
3. L. Murphy, K. Huxley, A. Wilding, C. Robinson, Q. Foucart and L. Willems, *Chem. Sci.*, 2023, **14**, 5062-5068.