Photocatalytic Proximity Labelling to Probe Cell Surface Glycan Interactomes

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Introduction

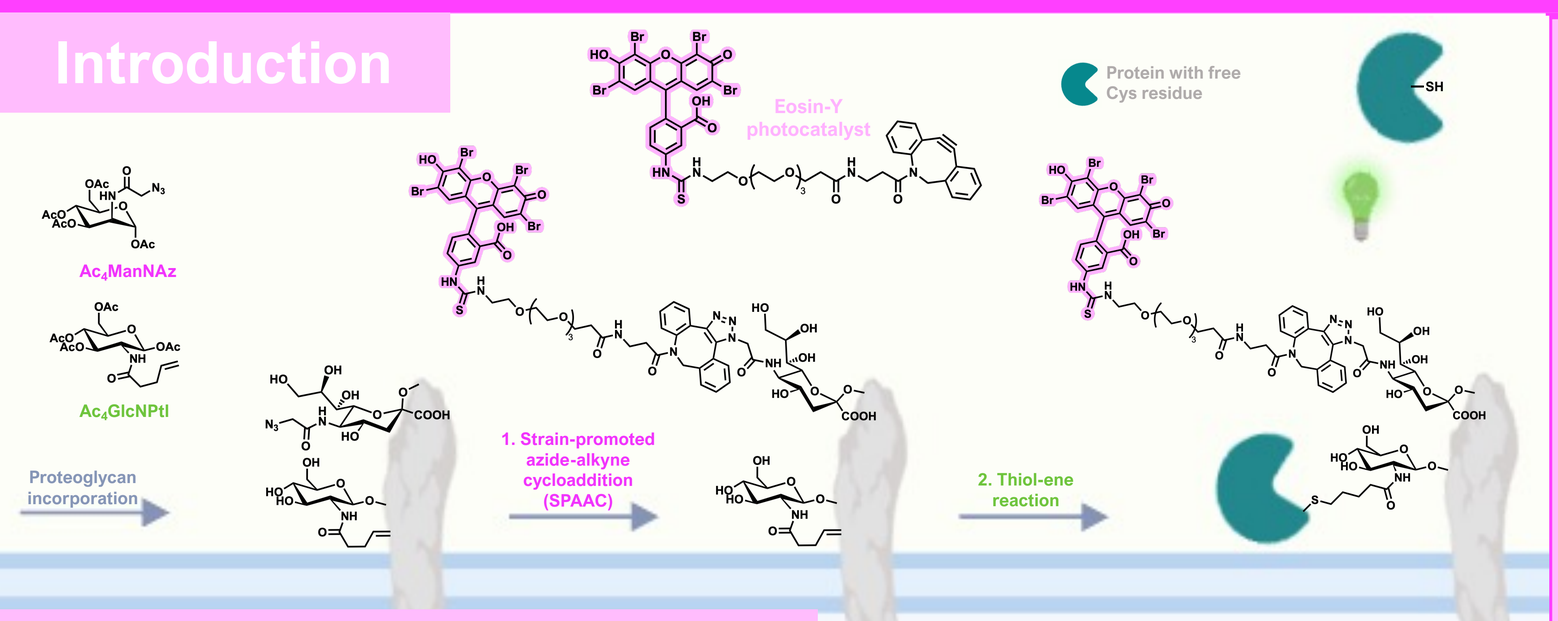


Fig. 2 Photocatalytic proximity labelling approach involving the installation of a cell surface catalyst, Eosin-Y, via SPAAC, followed by the thiol-ene trapping of Cys-containing proteins of interest.

Metabolic glycome labelling (MGL) is a powerful approach for investigating glycan dynamics during biological processes, including embryonic development and disease progression. The technique relies on the incorporation of chemically modified monosaccharide analogues into cellular glycans through endogenous biosynthetic pathways, followed by selective labelling using bioorthogonal reactions.[1]

This work presents the first application of thiol-ene chemistry in combination with strain-promoted azide alkyne cycloaddition (SPAAC) for the dual labelling of metabolically incorporated sugars. Thiol-ene reactions possess key bioorthogonal characteristics, including high selectivity, rapid kinetics, physiological stability, aqueous compatibility, and orthogonality to established click chemistry, yet remain underexplored in the context of MGL (fig. 1).[2]

Furthermore, incorporation of a covalently linked Eosin-Y photocatalyst enables spatially resolved cysteine interactome profiling through thiol-ene covalent trapping, followed by proteomic analysis (fig. 2).

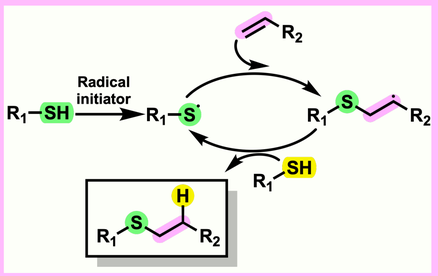


Fig. 1 The thiol-ene reaction mechanism

Thiol-ene Reaction In Vitro

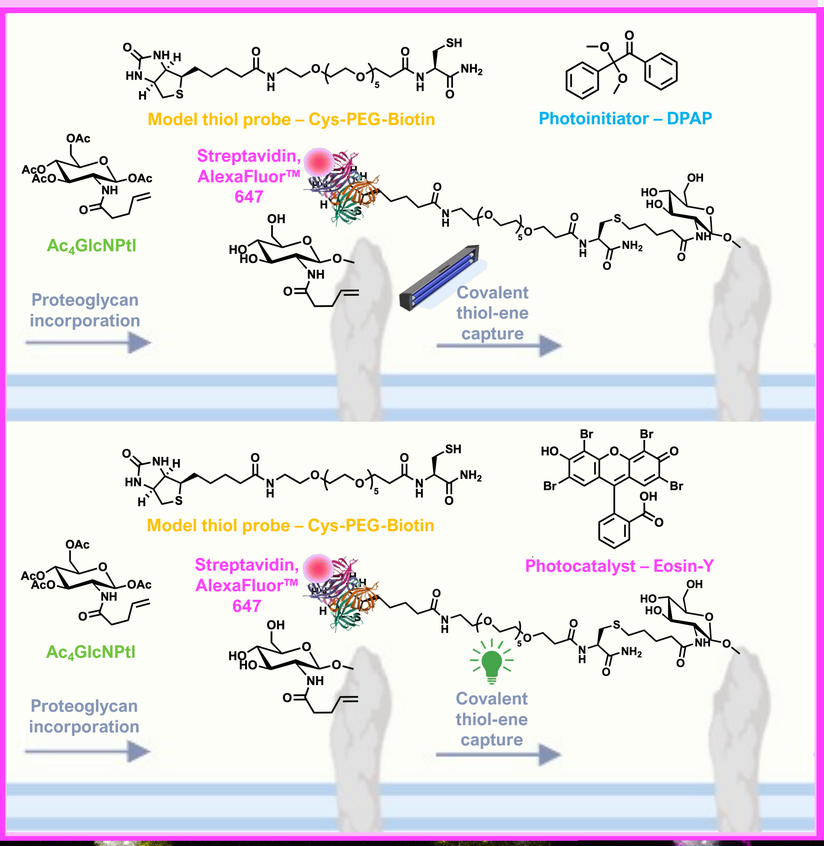


Fig. 3 HeLa cells were cultivated in the presence of $Ac_4GlcNPtl$ (50 μM) and incubated overnight at 37 °C and 5% CO_2. After washing with PBS, cells were treated with a thiol-ene cocktail (Cys-PEG-biotin (5 mM) and **DPAP (1 mM)** in 1% DMSO:PBS), irradiated at **354 nm** for 5 min, and then stained with streptavidin–AlexaFluor™ 647 prior to fixation, mounting, and imaging by confocal or STED microscopy.

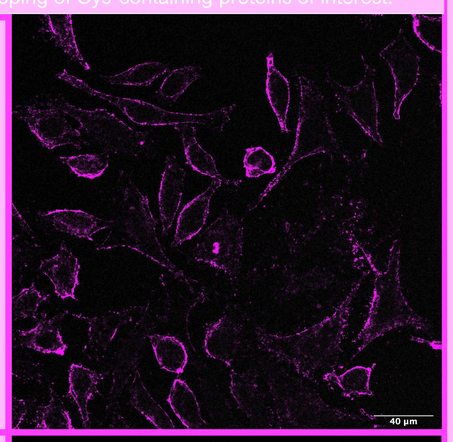
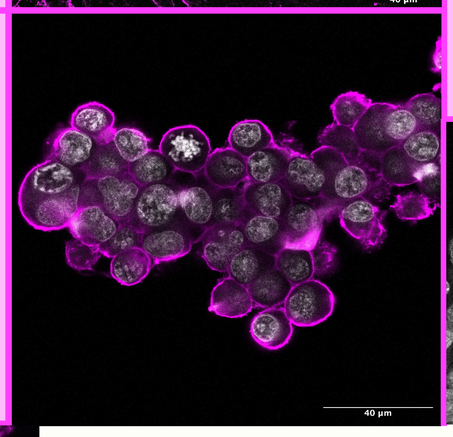


Fig. 4 HEK-293 cells were cultivated in the presence of $Ac_4GlcNPtl$ (50 μM) and incubated overnight at 37 °C and 5% CO_2. After washing with PBS, cells were treated with a thiol-ene cocktail (Cys-PEG-biotin (5 mM) and **Eosin-Y (7 μM)** in 1% DMSO:PBS), irradiated at **525 nm** for 10 min, and then stained with streptavidin–AlexaFluor™ 647 prior to fixation, mounting, and imaging by confocal or STED microscopy.



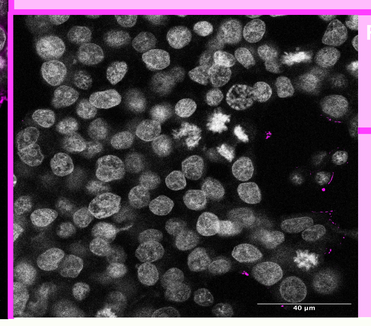


Fig. 5 Control HEK-293 cells incubated in the absence of $Ac_4GlcNPtl$

Dual Labelling via SPAAC and Thiol-ene

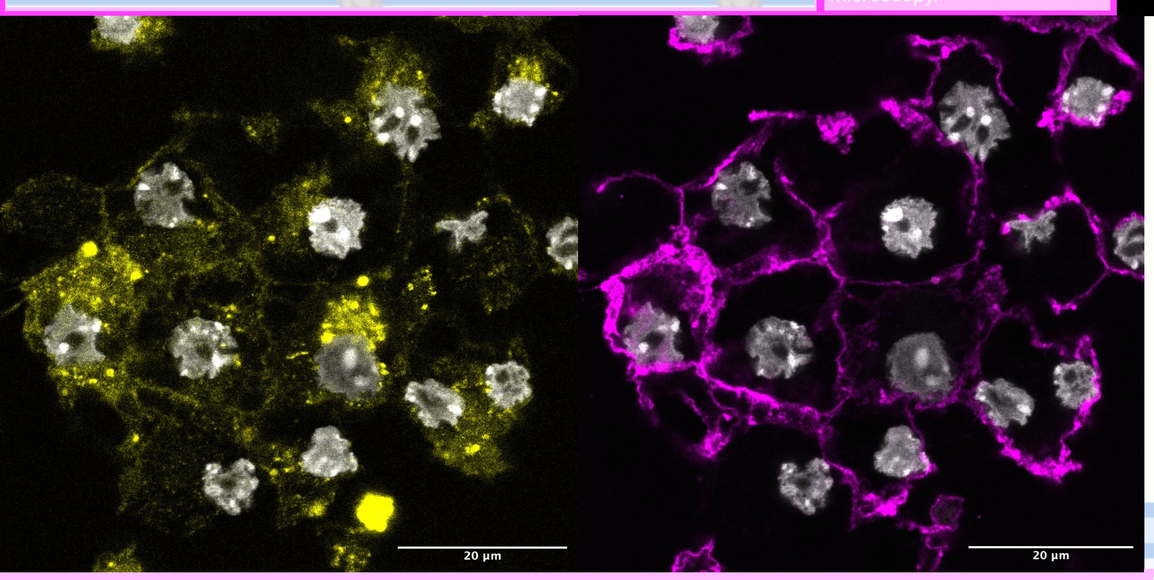


Fig. 5 Confocal images of dual labelled HEK-293 cells. Left: EY-PEG-DBCO photocatalyst installed on the cell surface, fluorescence detection ~ 550 nm. Right: Cys-PEG-Biotin probe installed on the cell surface, labelled with streptavidin, AlexaFluor ™ 647 for fluorescence detection, ~ 650 nm.

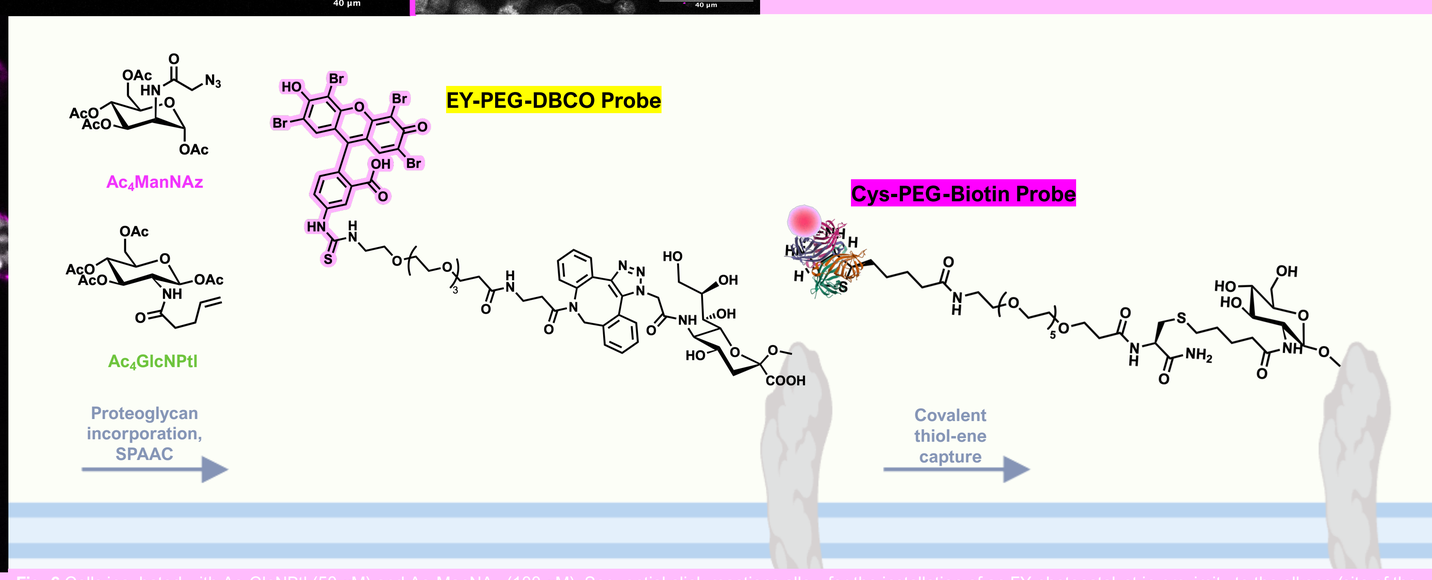


Fig. 6 Cells incubated with $Ac_4GlcNPtl$ (50 μM) and $Ac_4ManNAz$ (100 μM). Sequential click reactions allow for the installation of an EY photocatalyst in proximity to the alkene (site of the thiol-ene reaction, followed by the EY-mediated thiol-ene reaction to trap biomolecules/proteins containing a cysteine residue.

To expand the utility of the optimised thiol-ene approach, using Eosin-Y (EY) as a more biocompatible photocatalyst, a dual-labelling strategy was developed. This approached was inspired by the proximity labelling proteomics work developed by MacMillan and Wells, which involves the covalent linkage of a photocatalyst in proximity to a reaction site of interest.[3,4] An azide-functionalised sialic acid precursor ($Ac_4ManNAz$) was metabolically incorporated into cell surface glycans and subsequently labelled using an EY-PEG-DBCO probe via SPAAC. Following probe installation, the surface-bound EY catalysed the thiol-ene reaction, between a Cys-containing probe and the reactive alkene handle introduced through $Ac_4GlcNPtl$ incubation. This enables selective labelling of a second glycan population, and future iterations of this method would include Cys-containing proteins such as galectins and mucins. Dual fluorescence imaging successfully demonstrated simultaneous visualisation of both labelled glycan populations on HEK-293 cells (fig. 5), highlighting the potential of photocatalytic proximity labelling for multiplexed glycome analysis.

Conclusions and Future Work

Following the successful optimisation of the SPAAC, thiol-ene dual labelling strategy, future work will involve collaboration with Prof. Mia Huang, Scripps Research Institute, to elucidate cell surface glycan interactomes. Thiol-ene ligation will be used as a capture method to give a greater insight complex glycan-protein networks by overcoming the technical challenges associated with the inherently transient nature and low binding affinities of these interactions. Thiol-ene ligation offers superior temporal resolution in comparison to traditional analytical methods, and, as such, will be the current best-in-class method for mapping glycome landscapes.



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