Imaging Cell Surface Glycosylation *in vivo* using 'Double-Click' Chemistry

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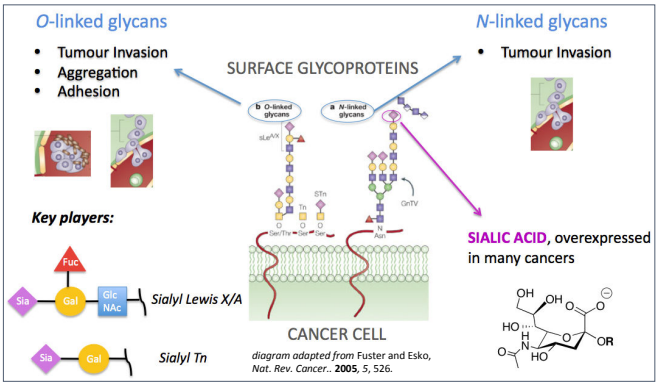
Introduction: Glycans in Cancer

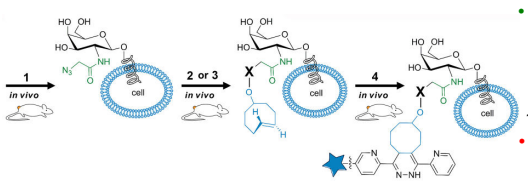


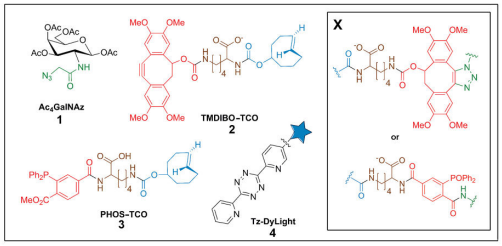
- Altered glycosylation on cell surface proteins is a key hallmark of tumour phenotype.

AIM: To develop new bioorthogonal probes for molecular imaging of glycan expression *in vivo*.

- Potential to translate to the clinic: Disease progression, response to treatment.

Metabolic Glycan Labelling





- $Ac_4GalNAz$: Peracetylated azido-labeled *N*-acetylgalactosamine is incorporated into *O*-linked surface glycoproteins.

1st CLICK REACTION

- TMDIBO: strained cyclooctyne that reacts with the azido glycan in a copper free Huisgen type [3+2] cycloaddition.

OR

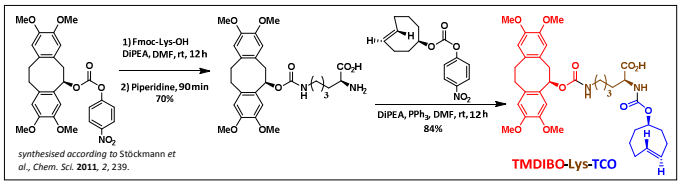
- Staudinger Phosphine (PHOS): reacts with the azido glycan *via* a Staudinger ligation.

2nd CLICK REACTION

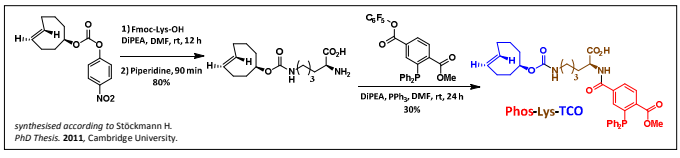
- *trans*-cyclooctene (TCO): reacts rapidly with fluorescent tetrazine in a [4+2] inverse electron demand Diels-Alder reaction.

'Double-click' Probes: Syntheses

➢ TMDIBO-TCO



➢ PHOS-TCO



In Vitro Cell Studies

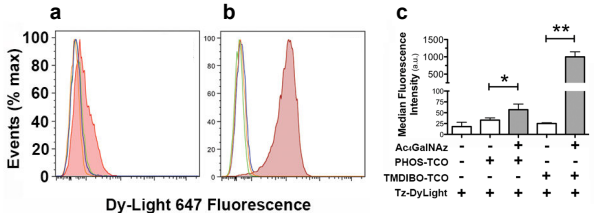


Fig. 1 Analysis of probe binding by flow cytometry. Lewis lung carcinoma (LL2) cells were cultured in the presence or absence of Ac4GalNAz (**1**) then incubated with either PHOS- TCO (**3**) or TMDIBO-TCO (**2**) and Tz-DyLight (**4**). Fluorescence histograms of LL2 cells labeled with PHOS-TCO (a) or TMDIBO-TCO (b). (c) Data are the mean±SD with n=3 replicate experiments/group (*P<0.05, **P<0.005). Control groups: green (cells); orange (cells + Tz-DyLight); blue (cells + PHOS-TCO or TMDIBO-TCO + Tz-DyLight; Test group: shaded red (cells + Ac4GalNAz + PHOS-TCO or TMDIBO-TCO + Tz-DyLight).

In Vivo Fluorescence Imaging of Glycans

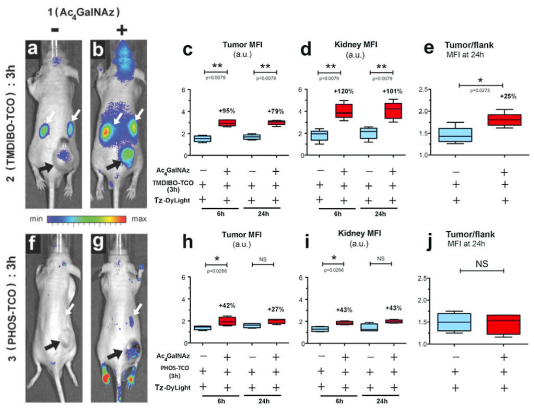


Fig. 2 Planar fluorescence images of live mice bearing subcutaneous lung (LL2) tumours, injected i.p. with solvent vehicle (a, f) or $Ac_4GalNAz$ (**1**) (b, g) daily for 3 days and then with TMDIBO-TCO (**2**) (a-e) or PHOS-TCO (**3**) (f-j). The fluorescent imaging agent, Tz-DyLight (**4**), was injected i.v. 3 h after injection of either (**2**) or (**3**). Fluorescence images (λ, excitation: 647 nm; λ, emission: 680 nm) were acquired at 6 h and 24 h after the injection of Tz-DyLight (**4**). Black and white arrows in (a-b, f-g) indicate tumours and kidneys, respectively. Data in (c-e, h-j) are mean ± S.E.M., N=5/group; box plots represent median and 95% confidence intervals. Differences were considered significant when P<0.05 (*) or P<0.005 (**). Data shown in (e) and (j) are the ratio of tumour median fluorescence intensity (MFI) to the adjacent control (flank) tissue MFI at 24 h post-injection of Tz-DyLight (**4**).

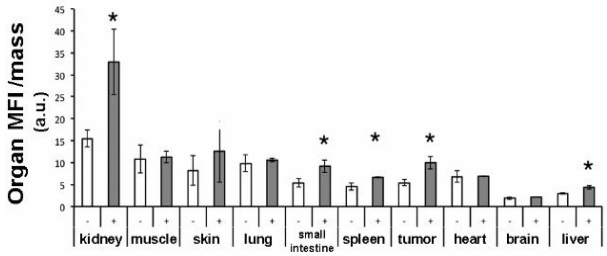


Fig. 3 Median Fluorescence Intensities (MFI) to mass ratios of metabolically labeled glycans in mouse tissues. Animals treated with solvent vehicle (-) or Ac4GalNAz (**1**) (+) as indicated, were then injected with TMDIBO-TCO (**2**) followed by Tz-DyLight (**4**). Organs were collected 24 h after the Tz-DyLight treatment. The weight-corrected mean whole-organ fluorescence intensities, calculated from macroscopic fluorescent images collected using an IVIS200 camera, are shown for a panel of mouse tissues. Data are mean ± S.E.M.. Differences were considered significant when * P<0.05 (N=4-7/group). Error bars lie within the chart bars when not visible.

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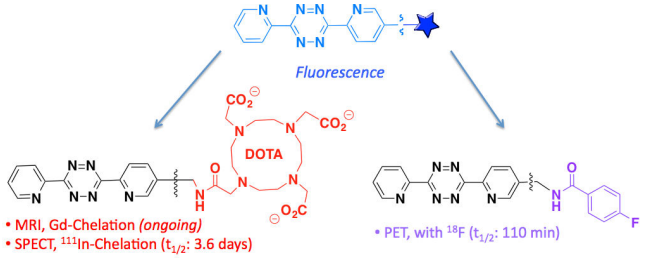
Conclusion and Future Perspectives

- 'Double click' probes for metabolic labeling: novel tools to study glycosylation *in vivo*.
- Probes showed lower non-specific binding to serum albumin proteins than previous probes (not shown in poster), enhancing contrast.
- Better tissue penetration and less non-specific retention *in vivo* enabled glycan metabolic imaging of highly glycosylated normal tissues.

Towards Clinical Imaging Modalities



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