

Protein conjugates in a flash: light-activated ortho-nitrobenzyl alcohols for the production of antibodies against cancer

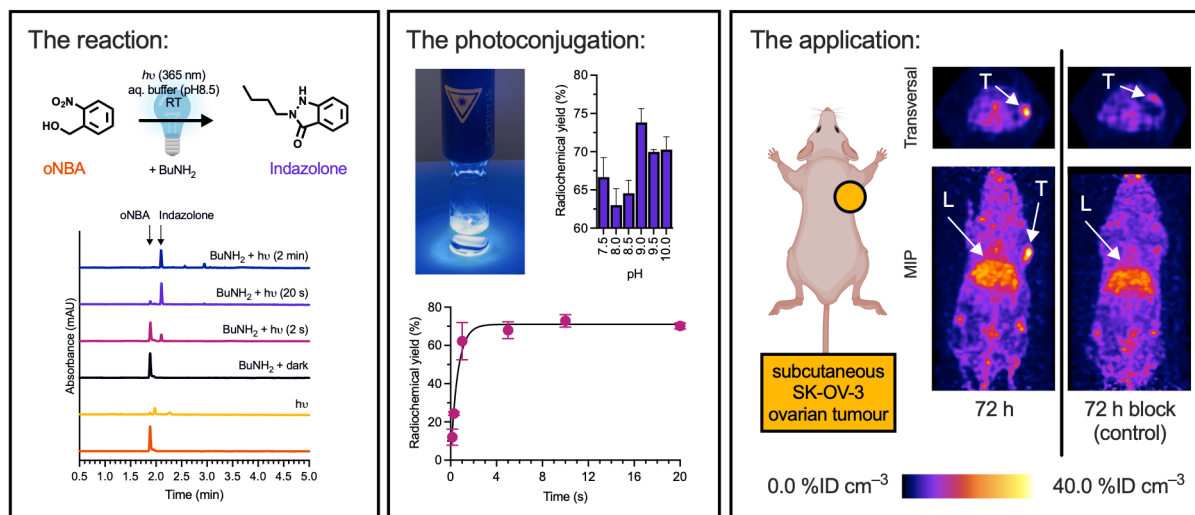
James W Southwell¹, Cesare Berton¹, Samy Kichou¹, Titouan Chetot¹, Rayk Schmitz, Jason P Holland*¹

¹ University of Zurich, Zurich, Switzerland

In cases where their receptor is over-expressed in tumor tissue, the incredible specificity of monoclonal antibodies (mAbs) can be exploited for the targeted delivery of radionuclides for the imaging and treatment of cancer. Here, we report a versatile photochemical conjugation platform¹ using an ortho-nitrobenzyl alcohol (oNBA) motif² that forms a bioconjugate bond under mild, aqueous conditions and with excellent radiochemical conversions (RCC), 76.4±4.6% and isolated decay-corrected radiochemical yields (RCY) 76.1±9.9% over a range of proteins and radionuclide combinations (n = 6). Photoactivation of oNBA proceeds at 365 nm with high conversion in seconds, to a reactive intermediate that forms an indazolone conjugate bond through reaction with accessible lysine side-chains on the protein surface.

Whilst initial experiments showed that the photochemical reactivity of oNBA with a lysine mimic gave near quantitative conversion after ~20 s under purely aqueous conditions, the reaction was even faster on protein (~4 s). Next, a desferrioxamine-oNBA (DFO-oNBA) chelator was synthesised and quantitatively radiolabelled with the PET imaging radionuclide, zirconium-89. This complex was investigated for the radiosynthesis of [⁸⁹Zr]Zr-DFO conjugates with trastuzumab and onartuzumab for imaging ovarian and gastric cancer models, respectively. Both mAb conjugates retained excellent serum stability and immuno-competency *in vitro*. Subsequent imaging and biodistribution studies with [⁸⁹Zr]ZrDFO-indazolone-trastuzumab on immunocompromised athymic nude mice bearing sub-cutaneous SK-OV-3 xenografts showed excellent tumour uptake (23.9±2.8 %ID g⁻¹) and specificity (~72% tumour uptake reduction in blocking experiments). The onartuzumab conjugate also provided excellent imaging, where use of the aza-macrocyclic DOTA-oNBA chelator facilitated a therapy study using the beta-emitting radionuclide terbium-161.

This work establishes oNBA as a robust photoactive handle for developing diagnostic and therapeutic protein conjugates. We continue to explore use of the oNBA motif in the synthesis of other biomolecule conjugates to access novel pharmaceuticals and imaging agents.



Keywords: bioconjugation, radiochemistry, photosynthesis, monoclonal antibodies.

1. Berton et al. , Berton, C.; Holland, J. P.; Chem. Commun. **2025**, 61, 5234-5252
2. Guo, A.-D.; Wei, D.; Nie, H.-J.; Hu, H.; Peng, C.; Li, S.-T.; Yan, K.-N.; Zhou, B.-S.; Feng, L.; Fang, C.; Tan, M.; Huang, R.; Chen, X.-H. Nat. Commun. **2020**, 11 (1), 5472.