# Chemodivergent Manganese-Catalyzed C–H Activation: Modular Synthesis of Fluorogenic Probes

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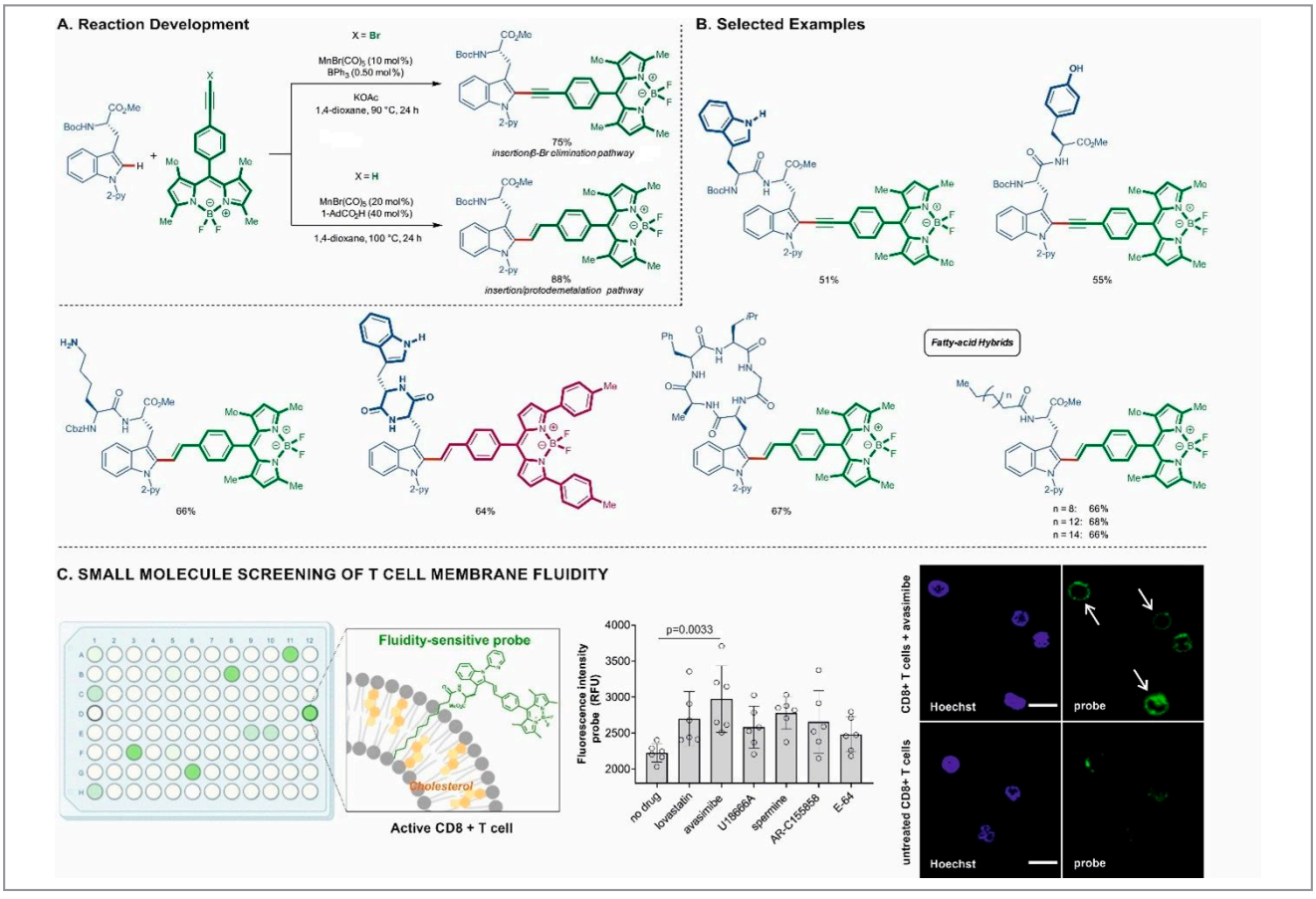
In the past few years, environmentally sensitive fluorophores have been developed to image cell-specific events associated to – among others – infection, inflammation and cancer in vivo. However, the vast majority of these strategies rely on elements of substrate pre-functionalization. As a consequence, there is increasing interest in developing new approaches for late-stage fluorogenic labeling of biomolecules with the aim of assembling molecular probes enabling for real-time imaging. In this context, the chemo- and positional-selective modification of biologically relevant compounds is particularly important for the late-stage diversification of biomolecules endowed with new spectral and biological capabilities.

The groups of Professor Lutz Ackermann from the Georg-August-Universität Göttingen (Germany) and Professor Marc Vendrell from The University of Edinburgh (UK) engaged in a successful collaboration aimed at advancing the state-of-the-art in the area of fluorogenic probes suitable for real-time in vivo imaging, which resulted in the publication of the title paper in *Nature Communications*. Professor Ackermann said: "During the last decade, C–H activation has surfaced as an increasingly viable tool for molecular syntheses, with enabling applications to total syntheses, material sciences, medicinal chemistry, and – very recently – chemical biology. In this regard, major efforts have been devoted to establishing C–H




**Scheme 1**

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functionalization of peptides with precious transition metals, such as palladium, while Earth-abundant 3d transition-metal catalysis continues to be scarce. Apart from being relatively inexpensive, 3d transition metals are generally less toxic and, more importantly, offer complementary reaction manifolds. Manganese complexes typically demonstrate low toxicity, thus their utilization in C–H functionalization of biomolecules is highly desirable. Moreover," continued Professor Ackermann, "manganese(I)-catalyzed C–H activation represents a mild, robust platform for transformative C–H activation manifolds. Thus, our strategy for a divergent assembly point for the preparation of fluorescent peptides with tunable optical properties identified internal bromoalkynes and terminal alkynes as viable substrates (Scheme 1A). We envisioned that we thereby could gain access to fluorescent imaging probes, in which the phenyl-BODIPY fluorescent core would be stitched to the indole moiety of a tryptophan amino acid via a linear alkynyl or a bent alkenyl spacer, while at the same time extending the conjugate π-system to fine-tune the fluorogenic behavior." The authors further wondered whether the nature of the linkage would not only alter the fluorescent properties of the peptide, but could also render fluorescent molecular rotors due to different rotation barriers. "Thus, within an insertion/β-elimination sequence, the desired BODIPY-labeled amino acids with a linear linkage were obtained," added Professor Ackermann. "In sharp contrast, 1-Ad$CO_2$H as additive enabled a divergent pathway via an insertion/protodemetalation manifold when terminal BODIPY-alkynes were used, thus providing the bent spacer. The mildness of our approach was reflected by an outstanding functional group tolerance for the late-stage C–H labeling of complex peptides (Scheme 1B)."

Professor Vendrell explained that the fluidity of cell membranes is an essential microenvironmental parameter for the proper function of a plethora of biological processes. "Thus, the study of changes and dysregulations on the composition of the plasma membrane is key from a biomedical perspective, from the fundamental study of disease mechanisms, such as cancer or Alzheimer's disease, to the translation of new therapeutics," said Professor Vendrell, who added: "Recent studies have established a direct correlation between the composition of the plasma membrane of T cells, which are key mediators of the adaptative immune system against infections and cancer, and their cytotoxic activity. These discoveries immediately caught our attention due to their potential use for the development of new fluorogenic turn-on probes as reporters of CD8+ T cells. Here, we used a rational design of different BODIPY molecular rotors to discover highly sensitive fatty acid-conjugated viscosity probes, which emit bright fluorescence when in contact with the membranes of T cells. This smart probe allowed us to image, in real-time, changes in the activation state of live human CD8+ T cells under physiological conditions."

The new fluidity-sensitive probe was used to establish a rapid fluorescence-based platform for the identification of small molecule modulators of CD8+ T cells (Scheme 1C). "Interestingly, among all drugs tested, cells treated with the Acyl-CoA:cholesterol acyltransferase inhibitor avasimibe exhibited the highest fluorescence emission," explained Professor Vendrell, adding: "Confocal microscopy experiments showed bright staining of the membranes in avasimibe-treated cells when compared to untreated cells." The team also confirmed the functional state of labeled CD8+ T cells by measuring the expression of receptor markers that are directly associated to immune activity. "This simple and cost-effective chemical platform to study immune responses could help in accelerating the design of more efficient immunotherapy treatments that invigorate the activity of CD8+ T cells in different diseases, including cancer," said Professor Vendrell.

"This project clearly demonstrates the strength of the combination of chemical, biological and medical studies, which allow the direct observation of cell-specific events. Furthermore, the successful collaboration between groups from different disciplines ensures that our discoveries not only have an immediate impact in the field on synthetic chemistry, but also in the area of biomedical sciences to tackle real-life problems," Professor Ackermann concluded.

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**Nikolaos Kaplaneris** was born in 1992 in Athens, Greece. He received his bachelor's degree in chemistry from the National and Kapodistrian University of Athens (Greece) in 2014. He obtained his master's degree in organic chemistry from the same university in 2016 following studies in the area of organocatalysis and photochemistry under the supervision of Prof. Christoforos G. Kokotos. In the same year, he joined the group of Prof. Lutz Ackermann at the Georg-August-Universität Göttingen (Germany) as a PhD student, working on late-stage peptide diversification and remote functionalization.

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**Marc Vendrell** graduated in chemistry at the University of Barcelona (Spain) in 2007. He then joined the Singapore Bioimaging Consortium to work in synthetic fluorophores for optical imaging. In 2012 he started his independent career as an academic fellow at the University of Edinburgh (UK) to develop and translate fluorescent peptide probes for imaging immune cells in humans. His research has led to several license agreements with industry to commercialise fluorescent probes worldwide (Trp-BODIPY, ApoTracker™ Green, SCOTfluors) and has been recognised with international awards and distinctions: SEQT Young Investigator Award (2007), SBIC Chairman's Prize (2010), ERC Consolidator Grant (2017), Fellow of the Royal Society of Chemistry (2017), Marcial Moreno Lectureship (2018) and SRUK Emerging Talent Award (2019). Since 2020, he is appointed as Chair of Translational Chemistry and Biomedical Imaging at the College of Medicine in Edinburgh.

*Prof. L. Ackermann*

**Lutz Ackermann** studied chemistry at the Christian-Albrechts-Universität Kiel (Germany) and obtained his Ph.D. in 2001 working with Prof. Alois Fürstner at the Max-Planck-Institut für Kohlenforschung in Mülheim/Ruhr (Germany). He was a postdoctoral fellow with Prof. Robert G. Bergman (University of California, Berkeley, USA) before initiating his independent research in 2003 at the Ludwig-Maximilians-Universität München (Germany), supported within the Emmy Noether Program of the Deutsche Forschungsgemeinschaft. In 2007, he became a full professor at the Georg-August-Universität Göttingen (Germany), where he served as the Dean of Research and Dean of Chemistry as well as the director of the Wöhler Research Institute for Sustainable Chemistry (WISCh). The development of novel concepts for homogeneous catalysis and their applications in sustainable organic synthesis, late-stage peptide diversification, and molecular imaging are among his main current research interests. His contributions have been recognized with awards such as the ERC Advanced Grant (2021), the Gottfried-Wilhelm-Leibniz-Prize (2017) and the ERC Consolidator Grant (2012). He has held various visiting and distinguished professorship positions in Huaqiao University (P. R. of China), Université de Strasbourg (France), École Polytechnique (France), IIT Bombay (India), Kyoto University (Japan), Università di Pavia (Italy) and Università degli Studi di Perugia (Italy).

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