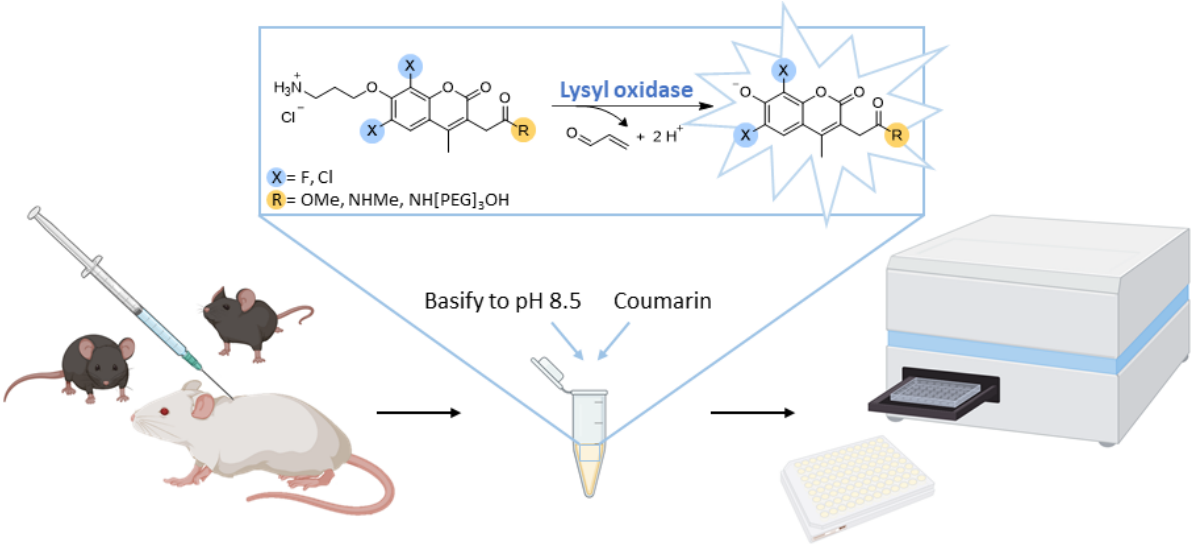
Evaluating Lysyl Oxidase Activity with Turn-On Fluorescent Probes

Laura M. Poller, Helma Wennemers
Laboratory of Organic Chemistry, ETH Zurich, Vladimir-Prelog-Weg 3, 8093 Zürich, Switzerland
laura.poller@org.chem.ethz.ch

Remodelling and maturation of collagen, the dominant structural protein in mammals, is crucial for the integrity of organs.[1,2] These processes include post-translational cross-linking of collagen strands triggered by the oxidation of lysine residues to reactive aldehydes through lysyl oxidases (LOXs). This enzyme family consists of five isoforms – lysyl oxidase (LOX) and four lysyl oxidase-like enzymes (LOXL1-4).[1] Excessive LOX activity is associated with fibrotic and malignant diseases which are estimated to account for around 45% of deaths in developed countries.[3] A comprehensive investigation of LOX activity is therefore important for a deeper understanding of normal physiological versus pathological processes. The current standard HRP assay detects hydrogen peroxide, the by-product of the oxidative deamination reaction, and lacks specificity.[4] Our group has recently developed an enzyme-reactive sensor that detects LOX *in vitro*, *in vivo* and in tissue sections.[5]



In this work, we developed a quick and straightforward assay for measuring LOX activity, based on the turn-on of a coumarin-based sensor. We screened a series of probe analogs and identified selective sensors for LOXL2 over related amine oxidases. The new probes allowed us to detect LOXL2 activity at nanomolar enzyme concentrations in serum and organ homogenates with a significantly higher signal-to-noise ratio compared to the current standard HRP assay. We anticipate that our tools will be valuable for the screening of drug candidates targeting LOXs and deciphering the role of LOXs' in healthy and fibrotic states.

References:

[1] S. D. Vallet, S. Ricard-Blum, *Essays Biochem.,* **2019**, *63*, 349-364.
[2] M. D. Shoulders, R. T. Raines, *Annu. Rev. Biochem.,* **2009**, *78*, 929-958.
[3] N. C. Henderson, F. Rieder & T. A. Wynn, *Nature,* **2020**, *587*, 555-566.
[4] F. Rodriguez-Pascual, T. Rosell-Garcia, *Anal. Biochem.,* **2022**, *639*, 114508.
[5] a) M. R. Aronoff, P. Hiebert, N. B. Hentzen, S. Werner, H. Wennemers, *Nat. Chem. Biol.,* **2021**, *17*, 865-871. b) P. Hiebert, G. Antoniazzi, M. Aronoff, S. Werner, H. Wennemers, *Matrix Biol.*, **2024**, *128*, 11-20.