

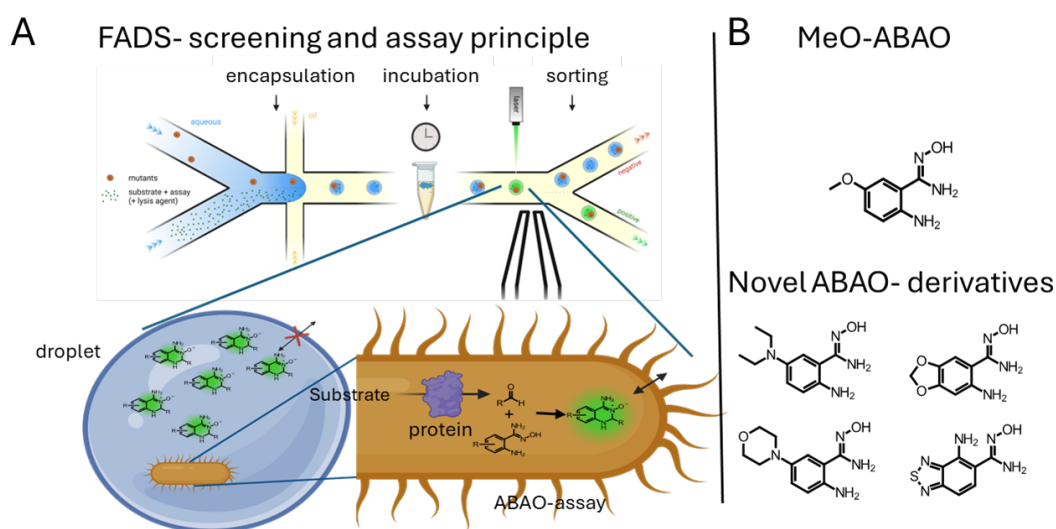
# Development of novel ABAO-based fluorogenic sensors for the application in a FADS system.

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Enzyme engineering in the form of random mutagenesis, is often strongly limited by the availability of suitable high-throughput screening. Fluorescence-activated droplet sorting (FADS), as one example, involves encapsulating single enzyme mutants in small water droplets within an oil phase, along with a chemical fluorogenic sensor and substrate.<sup>1</sup> Active mutants convert the substrate into a product, which is trapped by a fluorogenic sensor allowing detection and isolation of the active enzyme variants (Figure 1, **A**).



**Figure 1.** A) Principle of the FADS screening system and the ABAO assay. B) Methoxy-aminobenzamidoxime (MeO-ABAO) and novel ABAO derivatives.

For aldehyde-producing enzymes (e.g., aromatic dioxygenases), only a handful of chemical fluorogenic sensors are suitable for application in a FADS system due to the necessity of hydrolytic stability, high hydrophilicity, fast and selective adduct formation, and generation of a high fluorogenic turn-on. One promising starting point is based on the aminobenzamidoxime (ABAO) type assay with MeO-ABAO as the most utilized derivative (Figure 1, **B**).<sup>2</sup> It forms hydrolytically stable adducts upon the reaction with aldehydes which also display moderate fluorogenic properties. The exact mechanism by which the fluorescence emission is regulated has not been elucidated. Herein, we present our advancements in developing a FADS-compatible ABAO-based fluorogenic sensor and the first steps in the elucidation of the fluorescence mechanism. For this, some principal strategies of fluorophore optimization were utilized to design and test novel ABAO derivatives (Figure 1, **B**). One prominent candidate, the diethylamino-ABAO displayed promising characteristics compared to the respective MeO-ABAO adduct. The first results also indicate a push-pull type interaction in the fluorophore.

[1] M. Gantz, S. Neun, E. J. Medcalf, L. D. van Vliet, F. Hollfelder, *Chem. Rev.* **2023**, 123 (9), 5571-5611.

[2] A. K. Ressimann, D. Schwendenwein, S. Leonhartsberger, M. D. Mihovilovic, U. T. Bornscheuer, M. Winkler, F. Rudroff, *Adv. Synth. Catal.* **2019**, 361 (11), 2538-2543.