BIOTRANS
LA ROCHELLE 2023

N°1210 / PC
TOPIC(s) : Enzyme discovery and engineering

a high-throughput screening assay for alkane halogenases

AUTHORS
Hannes MEINERT / UNIVERSITY OF GREIFSWALD, FELIX-HAUSDORFF-STRASSE 4, GREIFSWALD
Christoffel P. S. BADENHORST / UNIVERSITY OF GREIFSWALD, FELIX-HAUSDORFF-STRASSE 4, GREIFSWALD
Hannah MEIER / UNIVERSITY OF GREIFSWALD, FELIX-HAUSDORFF-STRASSE 4, GREIFSWALD
Corresponding author : Uwe T. BORNSCHEUER / uwe.bornscheuer@uni-greifswald.de

PURPOSE OF THE ABSTRACT
Alkane halogenases are an interesting class of enzymes due to their importance in late-stage modification of organic molecules. However, a huge disadvantage is their limited substrate scope.[1]
We envisioned a concept for a high-throughput screening assay that could have the potential to drastically increase their substrate scope. The assay is based on the utilization of catalytically inactive haloalkane dehalogenases which bind their substrates irreversibly.[2] By utilizing these so called HaloTag proteins[3] in combination with a non-canonical fluorescent amino acid (flAA) and a fluorescent ligand a FRET effect can be employed to screen for halogenases that produce the desired haloalkane. In this context a positive signal would show the fluorescence of the flAA and a negative signal would be caused by the quenching of this fluorescence through the ligand which is added in a second incubation step (see Figure).
At this stage of the project a suitable combination of amino acid and fluorescent ligand that quenches the flAA through FRET were identified. Currently, a position in the catalytically inactive haloalkane dehalogenase for the introduction of the fluorescent amino acid is searched for. The incorporation at this position should not impair ligand binding but should also be as close as possible to the active site that optimal quenching upon ligand binding occurs.

FIGURES

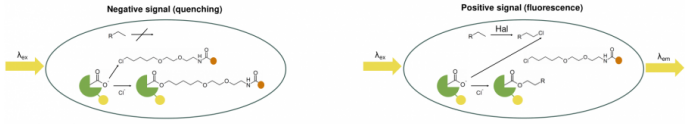

FIGURE 1

Assay Concept

The negative signal (left) results when no haloalkane is formed due to the absence of an active halogenase resulting in the quenching of the flAA (yellow) of the HaloTag (green) through the fluorescent ligand (orange). A positive signal (right) results fr

FIGURE 2

KEYWORDS

Alkane Halogenase | HaloTag | FRET | HTS Assay

BIBLIOGRAPHY

[1] Romero et al., Angew. Chem. Int. Ed., 2021, 60, 16824-16855

[2] Pries et al., J. Biol. Chem., 1995, 270, 10405-10411

[3] Marques et al., JACS Au, 2022, 2, 1324-1337