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High-throughput pH-shift assay for the screening of new PEP-dependent carboligase activities

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PURPOSE OF THE ABSTRACT
Neuraminic acid synthases (NeuS) are a family of PEP-dependent enzymes catalyzing an carbon-carbon bond formation, which is irreversible due to the concomitant release of phosphate. This renders these enzymes superior to related carboligases such as aldolases, which catalyze an equilibrium addition.[1] The identification of enzyme orthologs, or variants engineered by knowledge-guided smart mutagenesis, that are suitable for synthetic applications with non-native substrates requires a reliable high-throughput screening method.[2] With this aim, we have developed an assay that exploits the formal hydrolytic cleavage of the enol phosphate moiety with release of one equivalent of acid. The resultant drop in pH can be measured using a pH-sensitive dye such as Cresol Red, which changes its color from purple to yellow in an acidic environment.[3] This allows a semi-quantitative determination of the enzyme activity by reading the decrease in absorption at 570 nm.

The pH assay has been tested on both cell-free extract obtained by sonication or chemical lysis, confirming its applicability for large-scale library screening and avoiding lengthy purification processes. Lastly, the sensitivity of the pH dependent assay has been validated by measuring the activity of the neuraminic acid synthase from Neisseria meningitidis (NeuSnme.)[4] against its native substrate N-acetyl-D-mannosamine and structurally analogous sugars such as D-Mannose.

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FIGURES

FIGURE 1

FIGURE 2

KEYWORDS

High-throughput | Carboligation | Screening | Neuraminic acid synthase

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