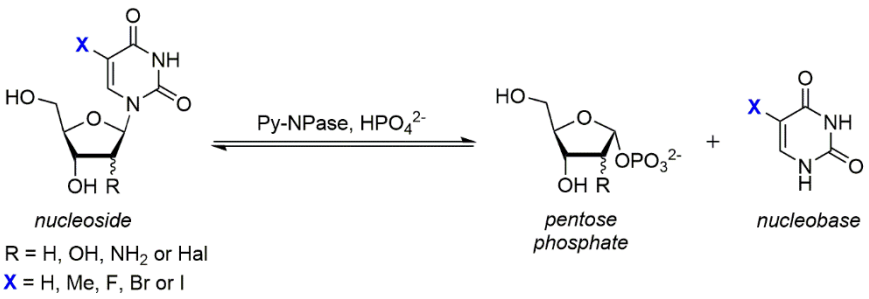
Kinetic and Thermodynamic Characterization of Nucleoside Phosphorylases

The Biocatalysis group of the Department of Bioprocess engineering at TU Berlin is offering a master project for a highly motivated student with an interest in biocatalysis.

Background

Nucleosides are highly functionalized biomolecules that enable encoding of information in DNA and RNA. Nucleoside phosphorylases are vital enzymes for nucleoside biosynthesis and degradation in all kingdoms of life and predominantly display specificity for either pyrimidine (Py-NPases) or purine nucleosides. Their use as biocatalysts in organic chemistry promises several inherent advantages over existing synthetic methods, such as aqueous reaction conditions that yield no toxic byproducts and do not require organic solvents or expensive reagents. Generally, they catalyze the reversible phosphorolytic cleavage of a nucleoside into the corresponding nucleobase and pentose-1-phosphate. Interestingly, substrate specificity and substrate functional group tolerance, both at the sugar and the nucleobase, vary greatly between bacterial and mammalian nucleoside phosphorylases. Thermostable bacterial Py-NPases have been shown to accept a variety of substrates and display excellent activity even under harsh reaction conditions. However, little is known about the influence of reaction pH on the activity towards non-natural substrates such as 5-halogenated pyrimidines and the transition state thermodynamics of this exciting biocatalytic equilibrium-controlled reaction system. We seek to shine light on the variables affecting the kinetics of an extremophilic Py-NPase with non-natural substrates and build a fundamental understanding of the catalytic thermodynamics with our recently established high-throughput UV-based enzyme assay.



nucleoside — *pentose phosphate* — *nucleobase*

R = H, OH, NH_2 or Hal

X = H, Me, F, Br or I

Project Aim

Explore the

1) reaction kinetics of a Py-NPase with modified substrates,
2) role of protonation states of the substrates,
3) pH-dependent substrate preferences of the enzyme and
4) transition state thermodynamics of phosphorolysis in this enzyme.

Approach

High-throughput kinetic characterization of a thermostable Py-NPase at different temperatures, with various substrates and at variable reaction pH.

Qualifications

Sound pipetting skills and an affinity towards data analysis are essential. Prior experience working with enzymes is optional. An interest in (bio)physical chemistry is a plus.

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