Karlsruhe Institute of Technology

Institute for Biological Interfaces
IBG-1 | Biomolecular Micro- and Nanostructures

Toward Reproducible Enzyme Modeling with Isothermal Titration Calorimetry

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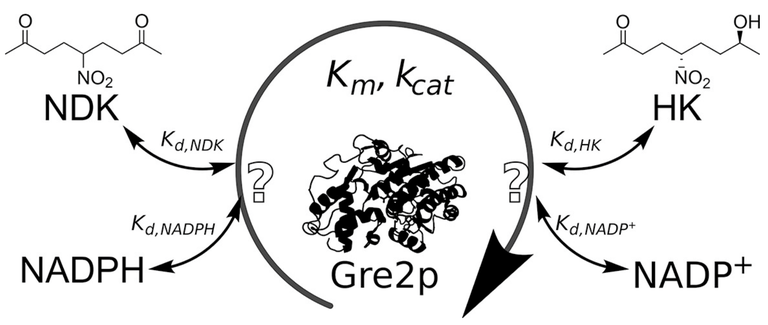
Relevance

To apply enzymes in technical processes, a detailed understanding of the molecular mechanisms is required. Kinetic and thermodynamic parameters of enzyme catalysis are crucial to plan, model, and implement biocatalytic processes more efficiently. While the kinetic parameters, K_m and k_{cat}, are often accessible by optical methods, the determination of thermodynamic parameters requires more sophisticated methods. Isothermal titration calorimetry (ITC) allows the label-free and highly sensitive analysis of kinetic and thermodynamic parameters of individual steps in the catalytic cycle of an enzyme reaction.

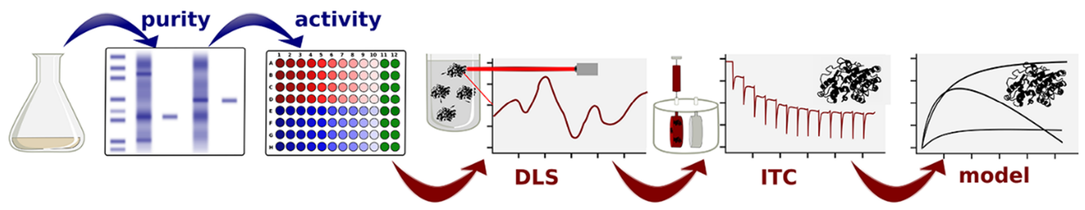
Aim

We aimed to established an ITC-dependent work flow to determine both the kinetic and the thermodynamic data for a NADPH-dependent enzyme Gre2p. This workflow should function as blueprint for future investigations of different enzymes. For this purpose, it is important to ensure reproducibility by the scientific community.



Methods

Traditional approaches to enzyme kinetic data (blue) primarily use spectrophotometric activity measurements. The implementation of DLS and ITC for quality control and mechanistic insights, respectively, leads to an increase in data quality to enable robust modeling.



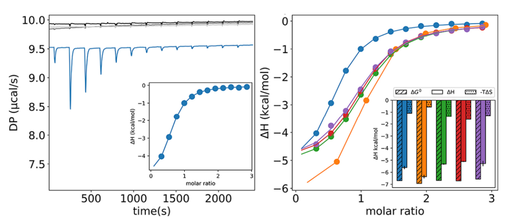
To achieve reproducibility, standard operating procedures (SOPs), data analysis and modeling workflows were published open and F.A.I.R. (findable, accessible, interoperable, and reusable).

Findings

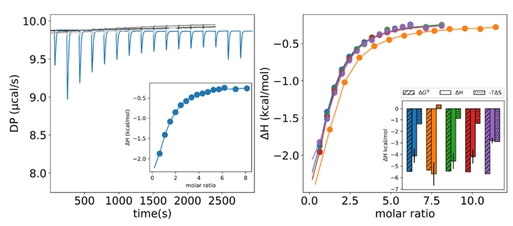
To untangle the reaction mechanism, traditional ITC binding experiments (ITC-BIND), multiple injection experiments (ITC-MIM) and single injection experiments (ITC-SIM) were performed.

ITC-BIND

NADPH



NADP⁺

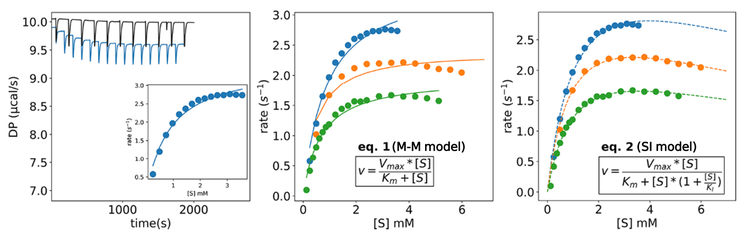


Ligand	K_d (µM)	ΔG^0 (kcal/mol)	ΔH (kcal/mol)	$-T\Delta S$ (kcal/mol)
NADPH	12.2 ± 2.5	-6.7 ± 0.1	-5.5 ± 0.5	-1.2 ± 0.4
NADP⁺	96.6 ± 18.0	-5.5 ± 0.1	-4.3 ± 1.0	-1.2 ± 1.2
NDK/HK	not measurable			

→ Higher affinity of NADPH to $Gre2p_{apo}$ than NADP⁺

→ NDK binding to $Gre2p_{apo}$ not observable

ITC-MIM



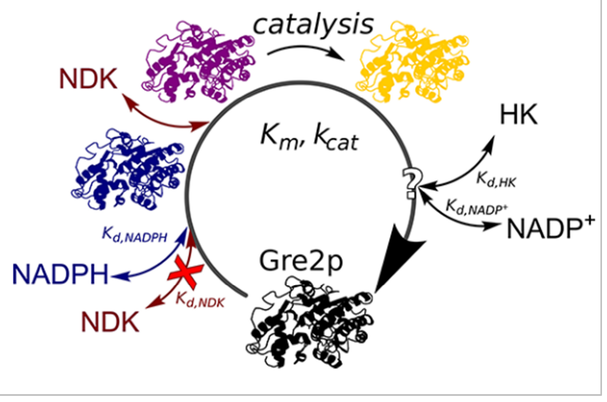
Model	Michaelis-Menten, eq. 1	Substrate inhibition, eq. 2
K_m (mM)	0.7 ± 0.2	1.4 ± 0.1
k_{cat} (s^{-1})	2.7 ± 0.8	3.6 ± 0.9
K_i (mM)	not applicable	10*K_m

→ Substrate inhibition model fits data better
→ K_m suggests NDK binding to $Gre2p_{holo}$ at least 10 times more favorable than to $Gre2p_{apo}$

Proposed mechanism

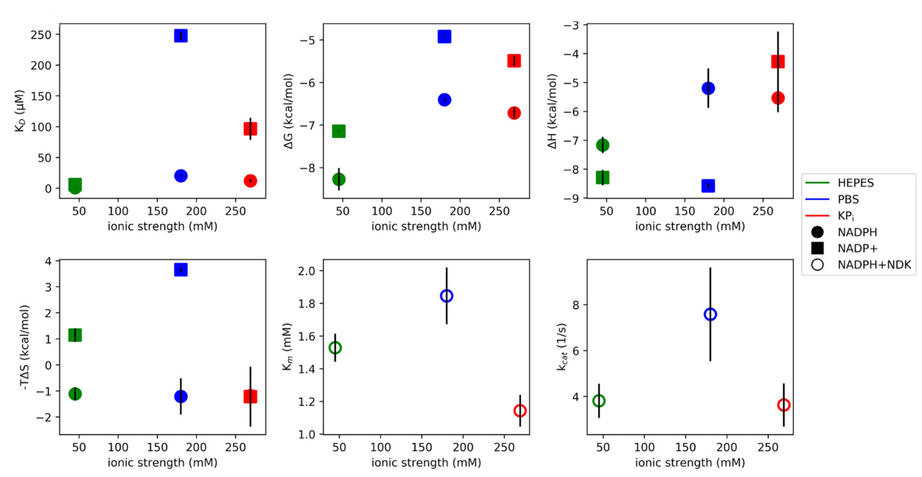
Ordered sequential:

- NADPH binds first to $Gre2p_{apo}$ (black)
- $Gre2p_{holo}$ (blue) has increased affinity to the substrate NDK which is represented in part by K_m
- Order of unbinding of HK and NADP⁺ cannot be determined experimentally



Impact of reaction buffer

Using different buffers changes the binding and kinetic parameters. Three buffers with low enthalpy of ionization of different ionic strengths were chosen for initial impressions.



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Sources

Publication: Ott F., Rabe K. S., Niemeyer C. M., Gygli G. (2021) Toward Reproducible Enzyme Modeling with Isothermal Titration Calorimetry, ACS Catalysis, 10695-10704.

Repository: Data, workflows and standard operating procedures are publicly available at fairdomhub.org.