Measuring Enzyme Kinetics, Inhibition, and Allostery using Isothermal Titration Calorimetry

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Enzymes in biology and disease

Many drugs act by modifying/inhibiting enzyme activity.

Enzymes can be therapeutics themselves

Methods for characterizing enzymatic catalysis and inhibition are important for designing drugs and understanding living things.


Enzyme inhibition

Rate of Catalysis

\[ \frac{1}{2} \frac{V}{V_{\text{max}}} \]

\[ \frac{1}{K_m} \]

\[ \frac{1}{V_{\text{max}}} \]

\[ \frac{1}{(V_{\text{max}})^2} \]

\[ \frac{1}{(K_m)^2} \]

\[ \frac{1}{(K_m - ESI)^2} \]

Substrate Concentration

Competitive

Uncompetitive

Noncompetitive

Mixed

EI

ESI

EI + ESI

$V_{\text{max}}$

$K_m$

$(V_{\text{max}})^{-1}$

$(K_m)^{-1}$

$(K_m - ESI)^{-1}$

$(Substrate\ Concentration)^{-1}$
Bi-substrate Reactions

The dependence of $K_m$ and $k_{cat}$ for one substrate on the concentration of the second substrate gives information on the kinetic mechanism.

Enzyme cooperativity

Energetic communication between active sites can lead to non-Michaelis Menten type behaviour.

\[ v_0 = \frac{V_{max} [S]^n}{(K_m)^n + [S]^n} \]

n = Hill Coefficient
Traditional methods to measure enzyme kinetics

Continuous Assays
- UV-Vis, fluorimetry, NMR
- Spectroscopically active reagents
- Coupled assays

Discontinuous Assays
- Chromatography, electrophoresis, MS
- Additional time, expense, uncertainty
Enzyme kinetics by Isothermal Titration Calorimetry (ITC)

ITC measures the heat released or absorbed in the sample cell during a titration as a function of time.
- Real-time readout of enzyme velocity.

Essentially universal enzyme assay
- Near-physiological conditions
- Spectroscopically-active reagents not required
- Compatible with opaque solutions
- Continuous assay, no ancillary techniques
- Multiple turnover – high sensitivity
- Direct readout of reaction rate – highly sensitive to changes in velocity
Enzyme kinetics by ITC

\[ \frac{dQ}{dt} = \text{enzyme velocity (mole/s)} \times \text{reaction enthalpy (cal/mole)} \]

\[ \frac{V_{\text{max}} [S]}{K_m + [S]} V_{\text{cell}} \]

\[ \Delta H \]

- heat flow (cal/s)
- enzyme velocity (mole/s)
- reaction enthalpy (cal/mole)

raw ITC output
kinetics and concentrations
integrate heat released by known amount of substrate
“Pseudo-first order assays”

Dilute enzyme: \( \frac{K_m}{k_{cat}[E]_0} \geq 10^4 \) s → catalysis does not affect [S]
Emerging view that kinetics (particularly residence times) are important for drug safety and efficacy.

- longer lifetimes lead to less sensitivity to fluctuations in drug and substrate concentrations.

Measuring structure kinetics relationships (SKR) in drug development is of growing interest.
Kinetics of inhibitor association and dissociation by ITC (prolyl oligopeptidase)

Kinetics of allosteric activator association (aminoglycoside phosphotransferase)
ITC detects enzyme velocity directly, unlike other methods which measure concentrations.

This makes it far more sensitive to changes in enzyme velocity.

Inhibitor/activator binding kinetics are clearly revealed.
"Continuous assays"

Read enzyme velocity ($V_0$) directly from the y axis.

Calculate substrate concentrations ([S](t)) from partial areas of peak.

$V_0$ versus [S] plot is obtained from a single ITC peak.

$[S](t) = \frac{\int_{t}^{\infty} P dt}{\int_{0}^{\infty} P dt}$

Strength & mode of inhibition (trypsin/benzamidine)

The benzamidine inhibitor accumulates in the cell with each injection.
Strength & mode of inhibition (trypsin/benzamidine)

Data agree with the competitive inhibition mode of trypsin/benzamidine.

Both mode and affinity are determined rapidly in a single, hour-long experiment, a 5- to 10-fold savings in time and material.
Bi-substrate enzymes (pyruvate kinase/PEP/ADP + Phe) 2D-ITC

\[ A + B \rightarrow P + Q \]
Pyruvate kinase/PEP/ADP + Phe

ADP in the cell is depleted with each injection.
Pyruvate kinase/PEP/ADP + Phe

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Two dimensional ITC

Michaelis Menten parameters ($k_{\text{cat}}$ and $K_m$) can be obtained from a single injection of substrate in under a minute.

ITC can very efficiently map how enzyme activity varies as a function of inhibitors (or product inhibition), activators, or other allosteric modulators.
Conclusions

ITC represents a universal and versatile tool for characterizing enzyme activity with accuracy and detail.