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

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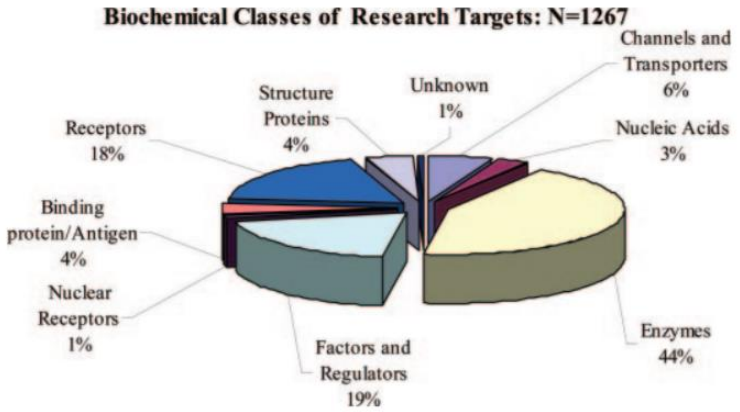
Higher Order Structure, April 14 2021

Enzymes in biology and disease

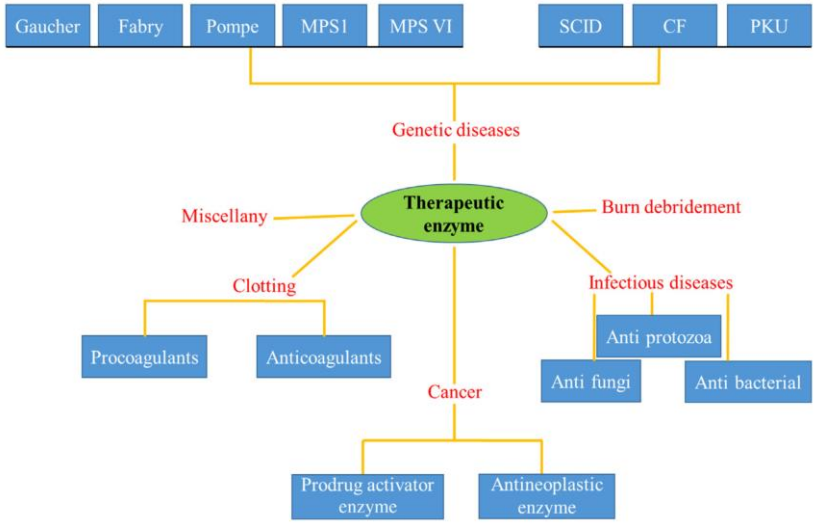
Many drugs act by modifying/inhibiting enzyme activity.

Enzymes can be therapeutics themselves

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

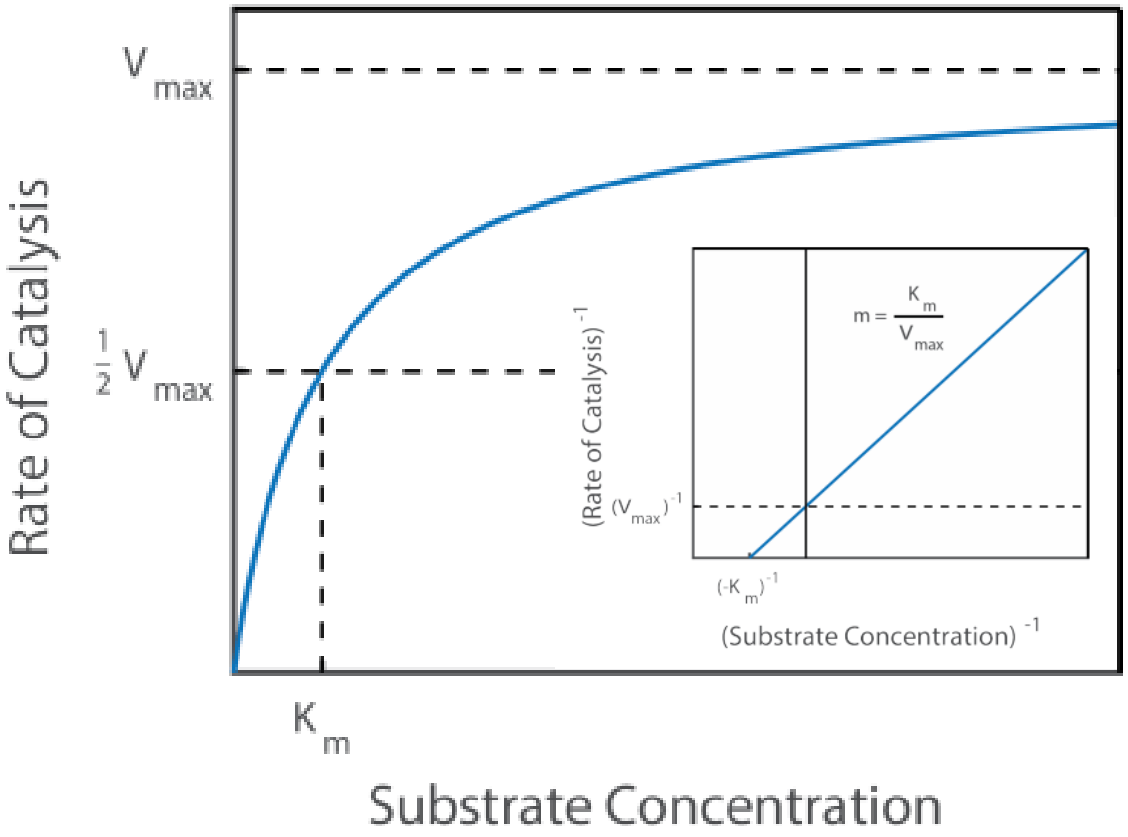


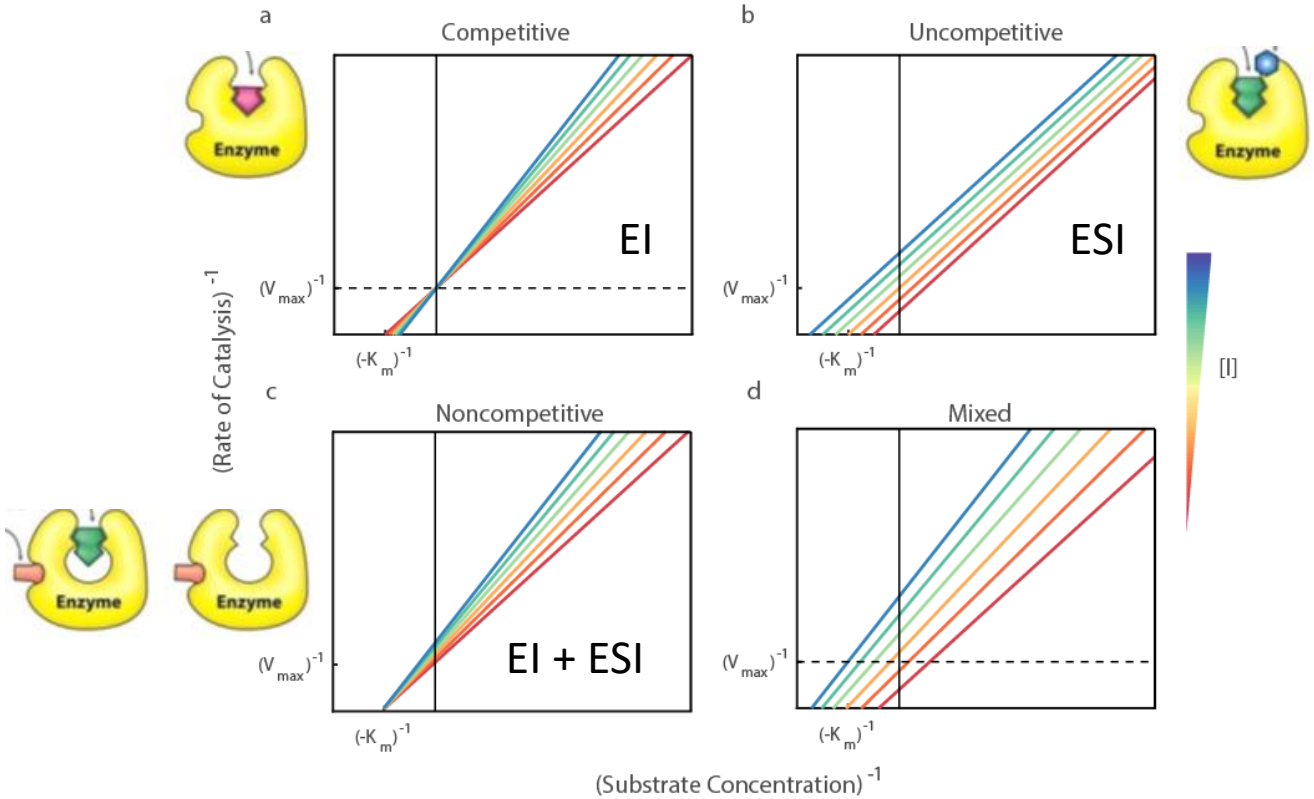
Therapeutic targets: progress of their exploration and investigation of their characteristics. Zheng CJ, Han LY, Yap CW, Ji ZL, Cao ZW, Chem YZ. Pharmacol Review 2006.



Application of therapeutic enzymes in different disorders and diseases. Kunamneni A, Ogaugwu, C, Goli D. Enzymes as therapeutic agents, Enzymes in Human and Animal Nutrition, Academic Press, 2018,

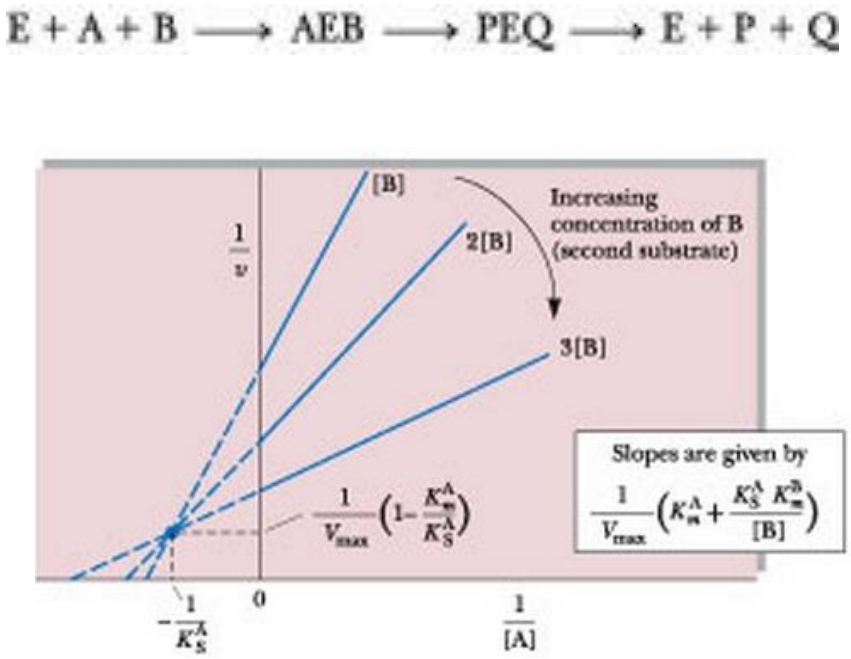
Enzyme inhibition

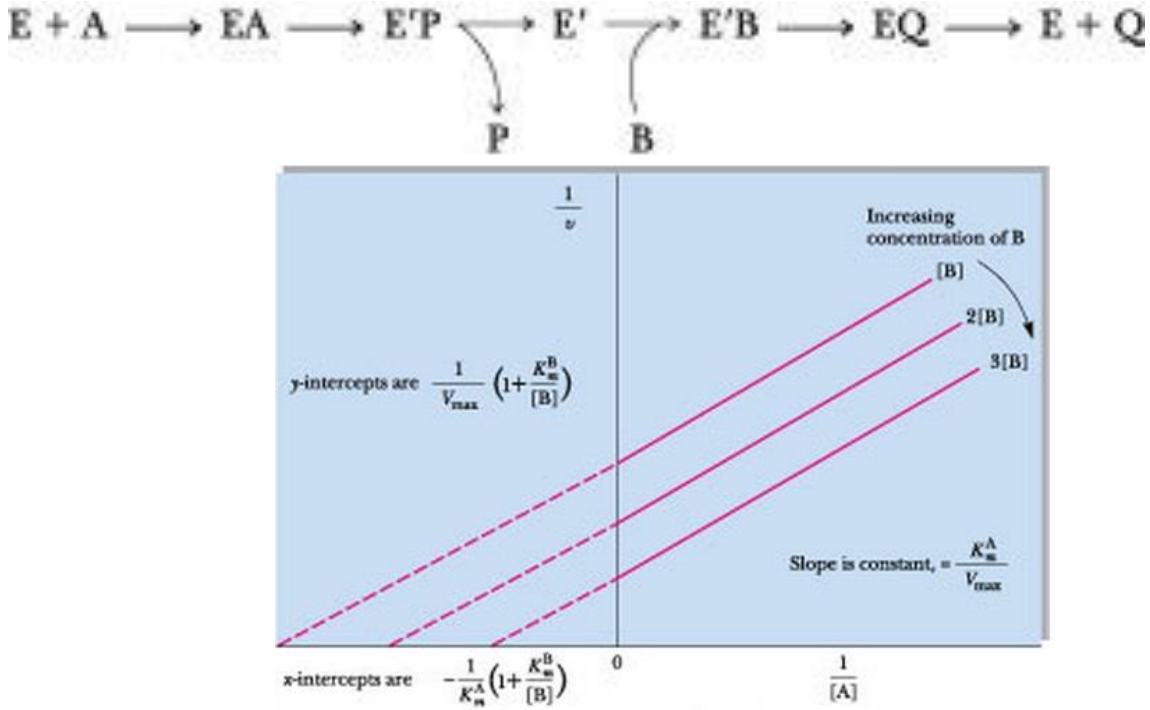




Bi-substrate Reactions

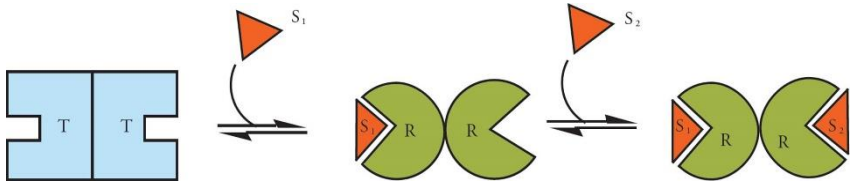
The dependence of K_m and k_{cat} for one substate on the concentration of the second substrate gives information on the kinetic mechanism.





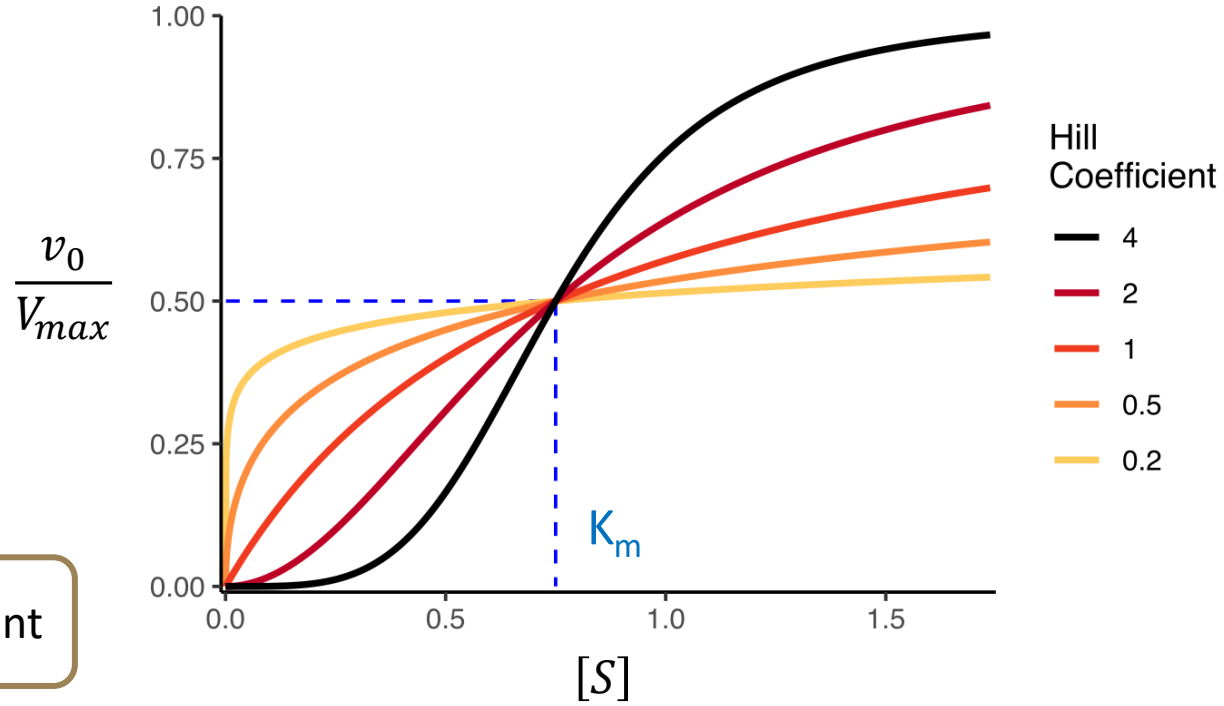
Enzyme cooperativity

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

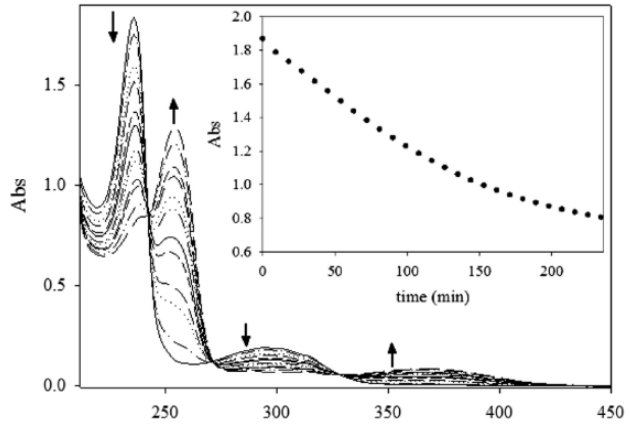


$$v_0 = \frac{V_{max}[S]^n}{(K_m)^n + [S]^n}$$

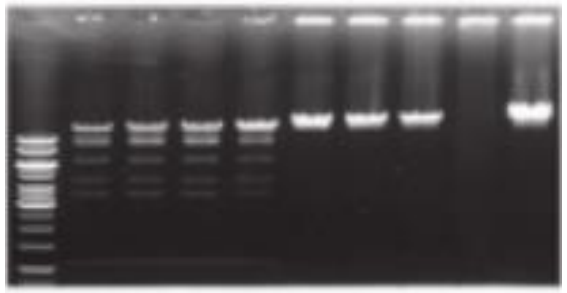
n=Hill Coefficient



Traditional methods to measure enzyme kinetics



Gluconjugates of 8-hydroxyquinolines as potential anti-cancer prodrugs. Valentina Oliveri et al. Dalton Transactions 2012



Large negatively charged organic host molecules as inhibitors of endonuclease enzymes. Tauran Y, Anjard C, Kim B, Rhim M, Coleman AW. Chem Comm 2014.

Continuous Assays

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

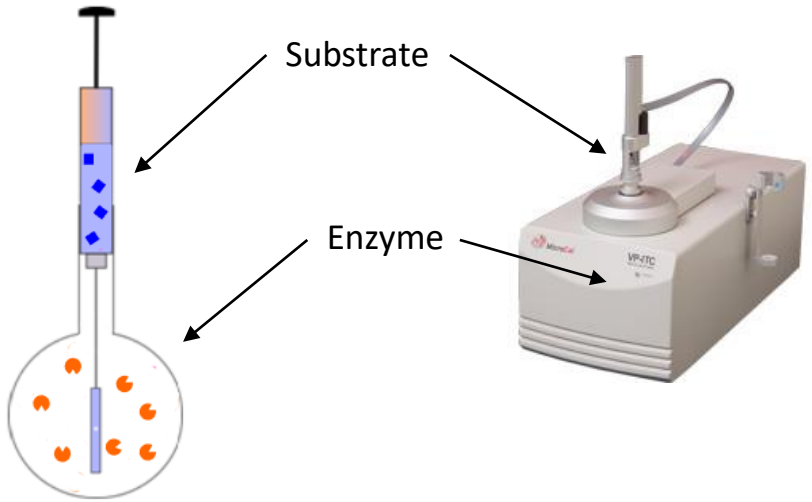
Discontinuous Assays

- 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.

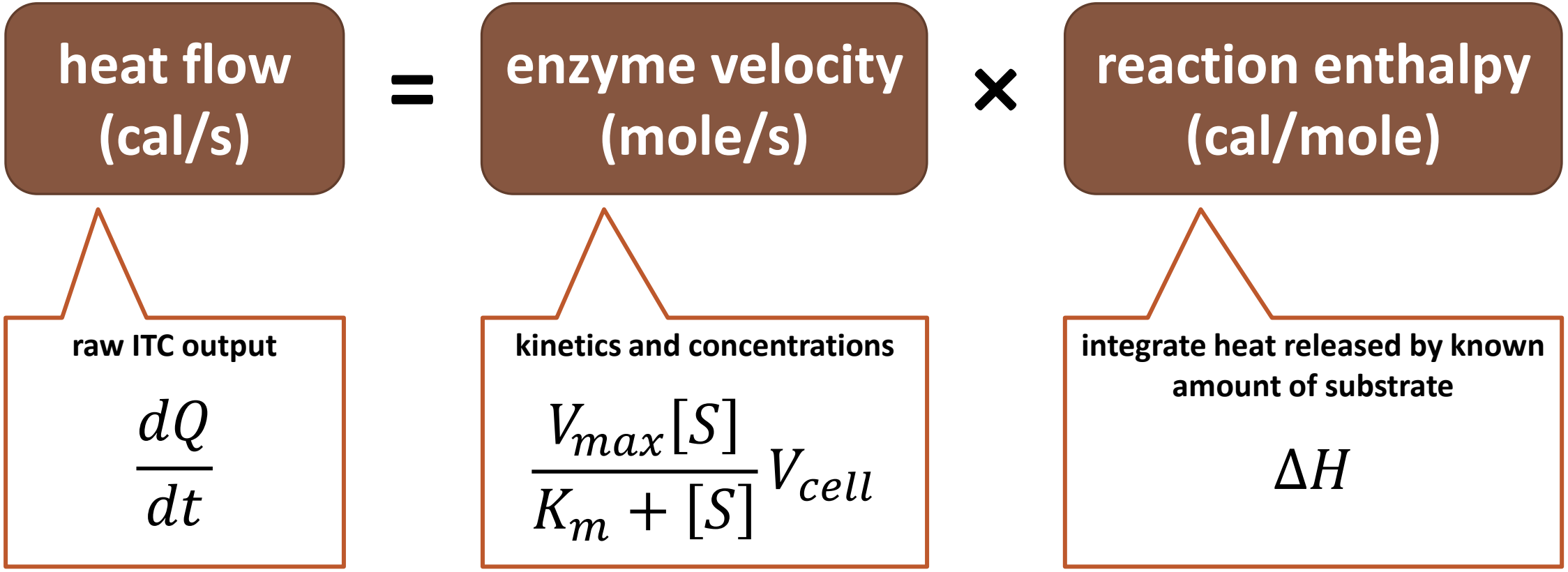
- Real-time readout of enzyme velocity.



Essentially universal enzyme assay

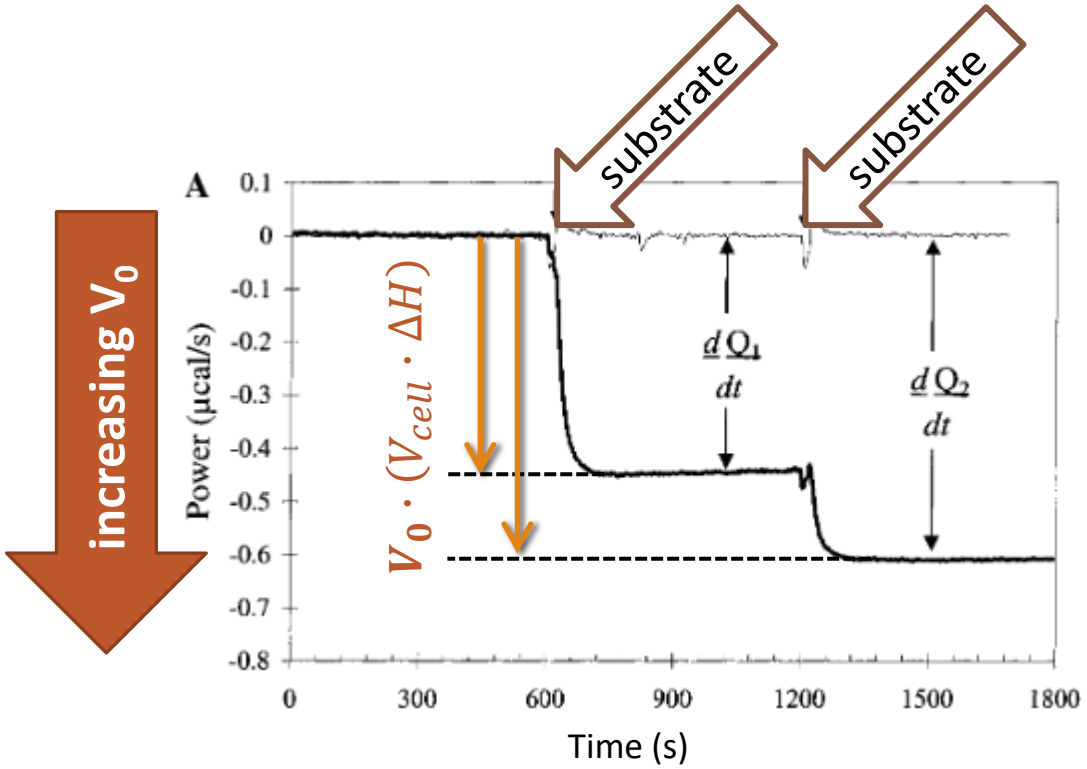
- Near-physiological conditions
- Spectroscopically-active reagents not required
- Compatible with opaque solutions
- Continuous assay, no ancillary techniques
- Multiple turnover – high sensitivity
- Direct readout of reaction rate – highly sensitive to changes in velocity

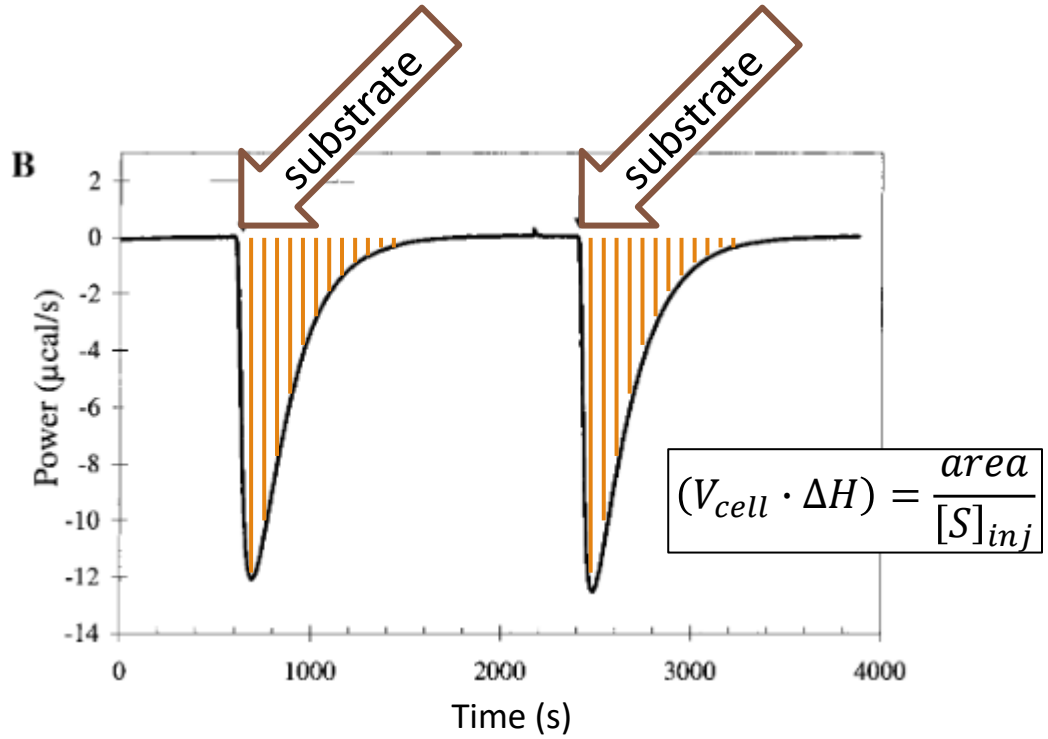
Enzyme kinetics by ITC



“Pseudo-first order assays”

Dilute enzyme: $\frac{K_m}{k_{cat}[E]_0} \geq 10^4$ s $\rightarrow$ catalysis does not affect [S]



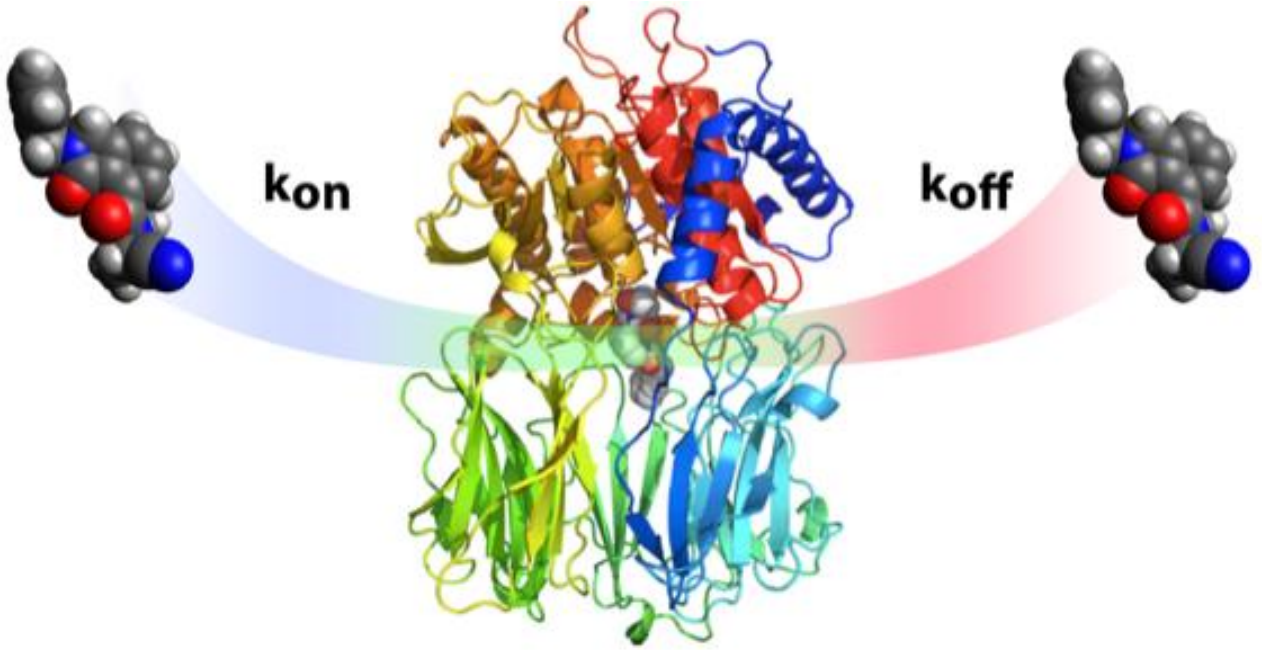


Structure Kinetics Relationships

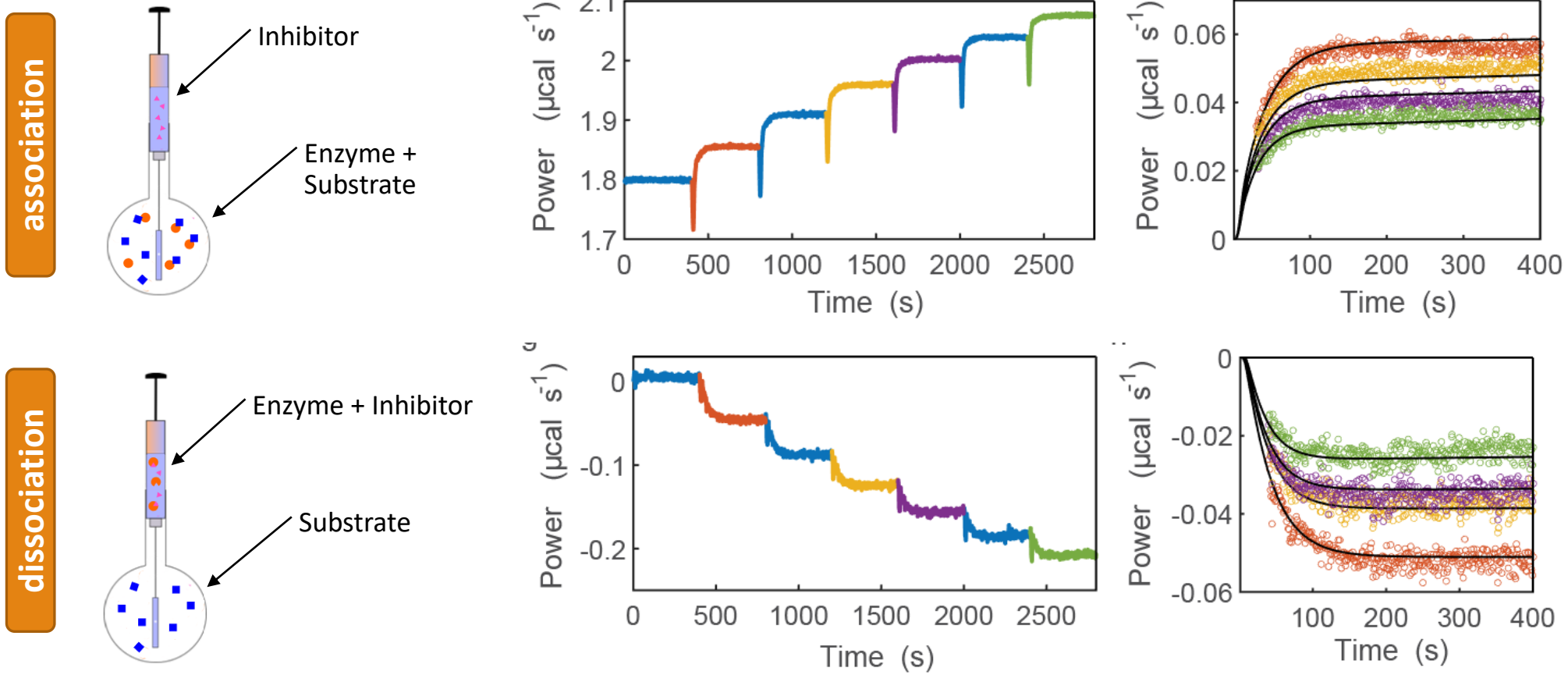
Emerging view that kinetics (particularly residence times) are important for drug safety and efficacy.

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

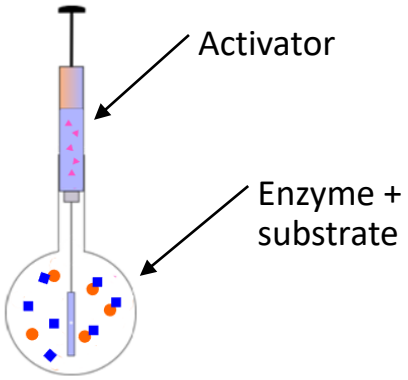
Measuring structure kinetics relationships (SKR) in drug development is of growing interest.

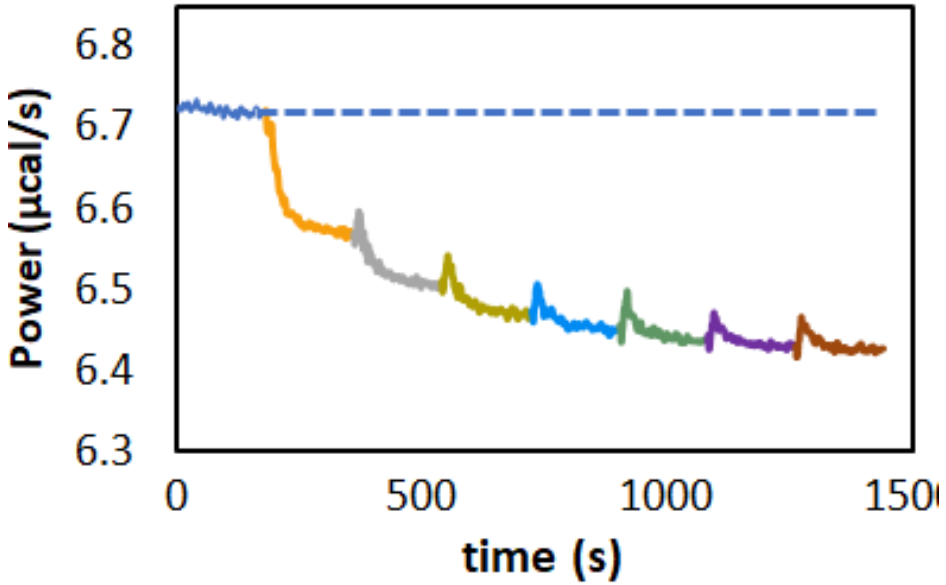


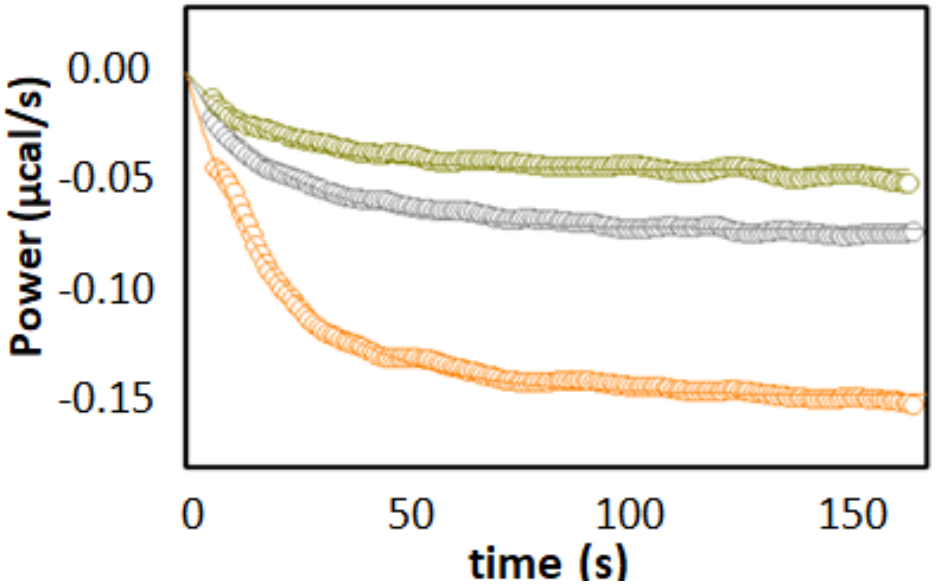
Kinetics of inhibitor association and dissociation by ITC (prolyl oligopeptidase)



Kinetics of allosteric activator association (aminoglycoside phosphotransferase)







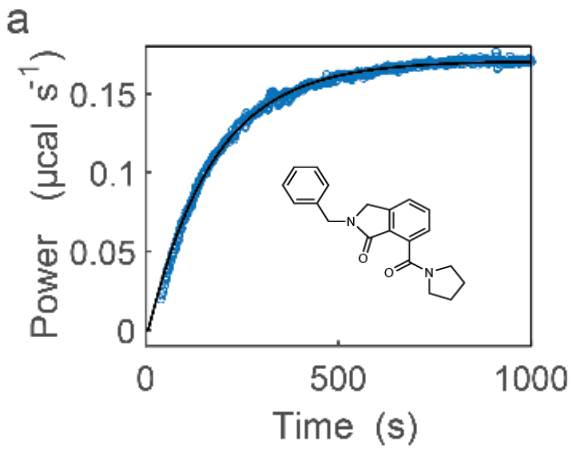
Functional binding kinetics by ITC

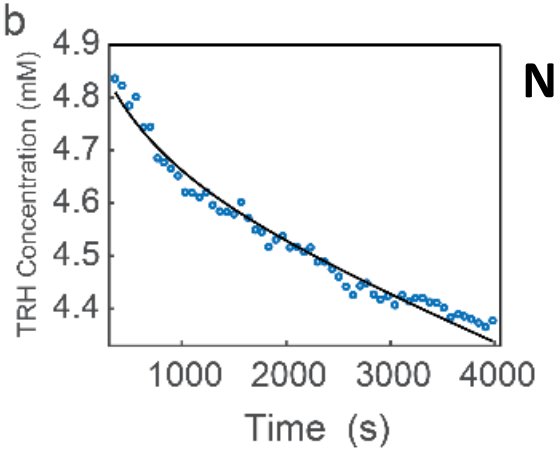
ITC detects enzyme velocity directly, unlike other methods which measure concentrations.

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

Inhibitor/activator binding kinetics are clearly revealed.

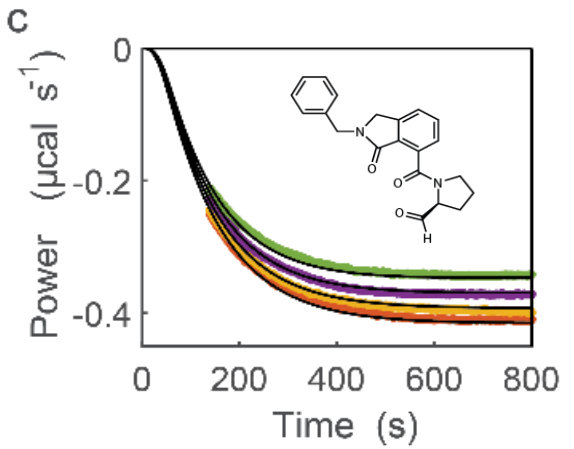
ITC k_{on}

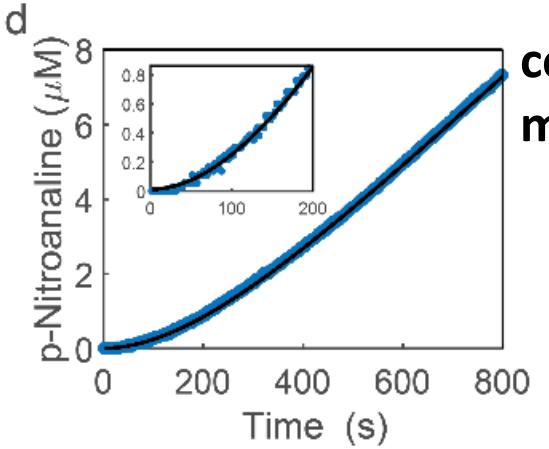




NMR

ITC k_{off}





colori-metric

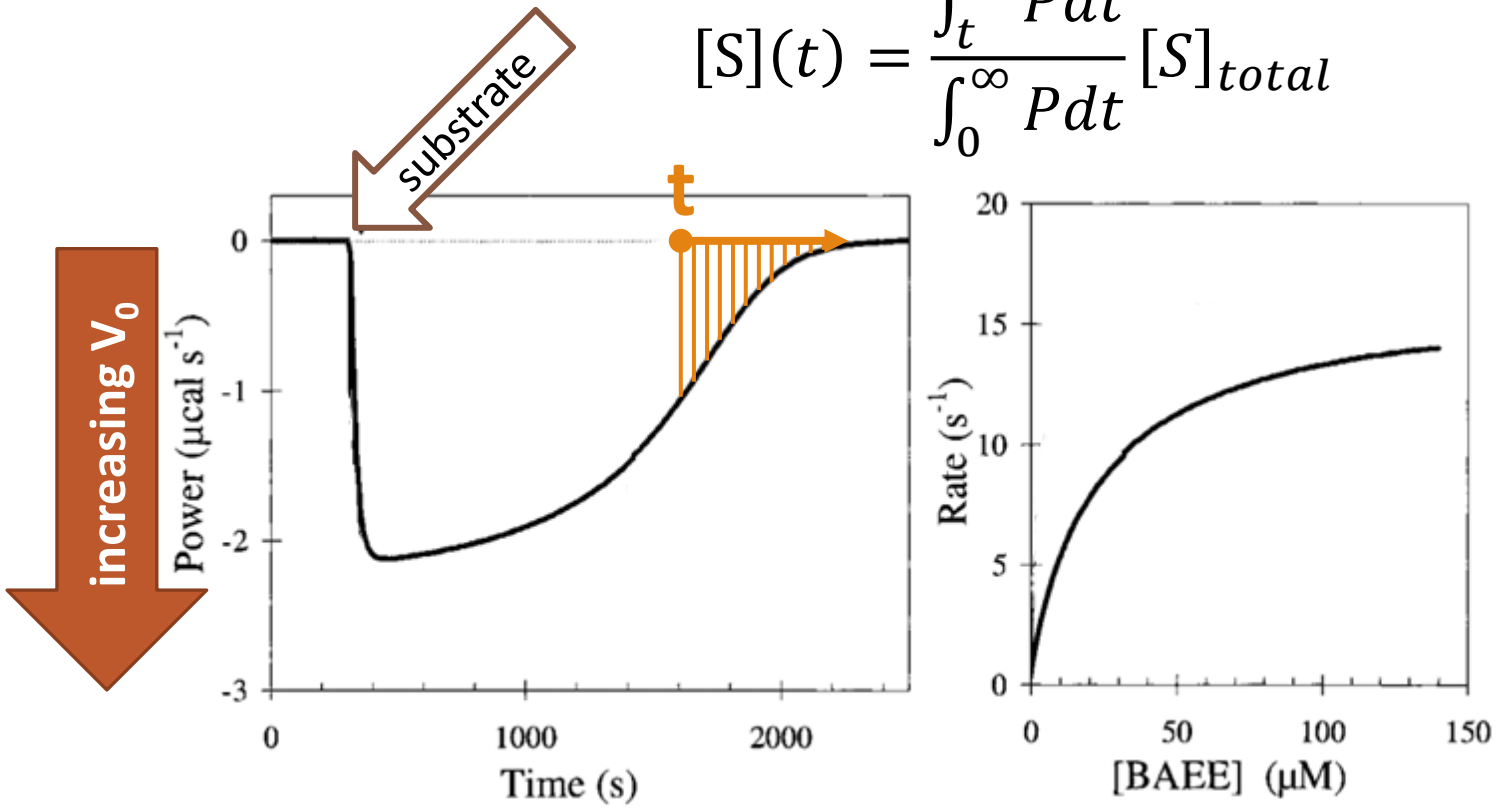
“Continuous assays”

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

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

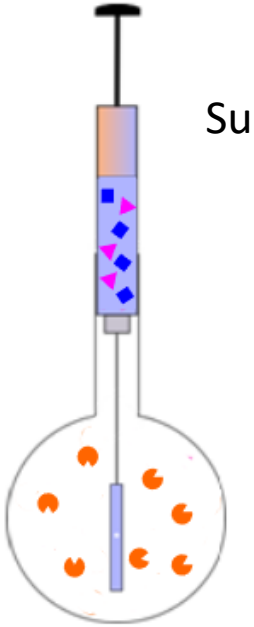
V_0 versus [S] plot is obtained from a single ITC peak.

$$[S](t) = \frac{\int_t^\infty Pdt}{\int_0^\infty Pdt}[S]_{total}$$



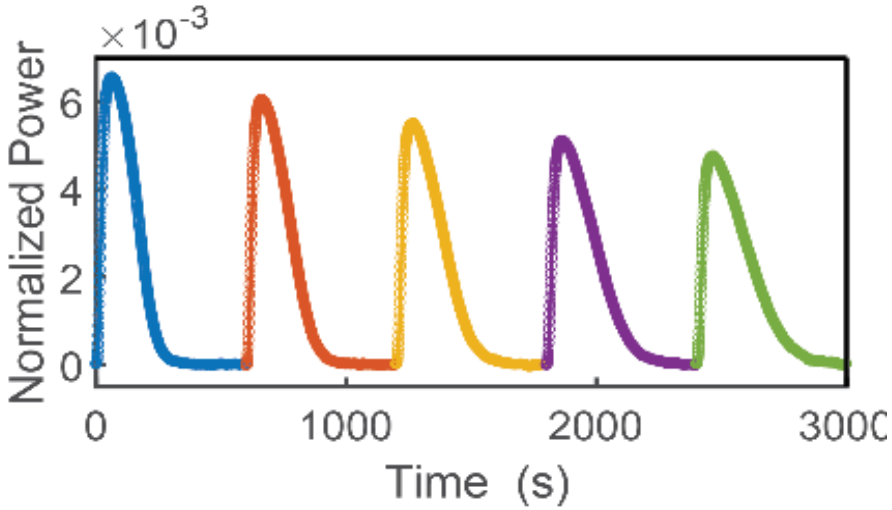
Strength & mode of inhibition (trypsin/benzamidine)

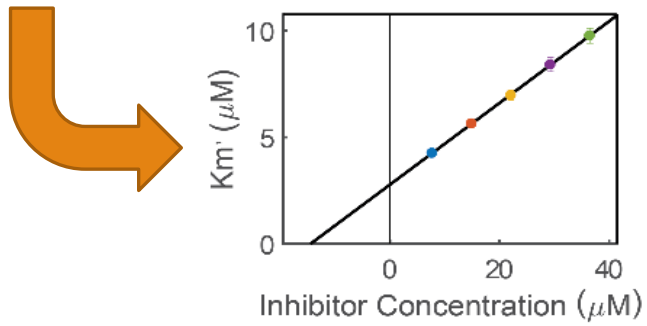
The benzamidine inhibitor accumulates in the cell with each injection.



Substrate + Inhibitor

Enzyme

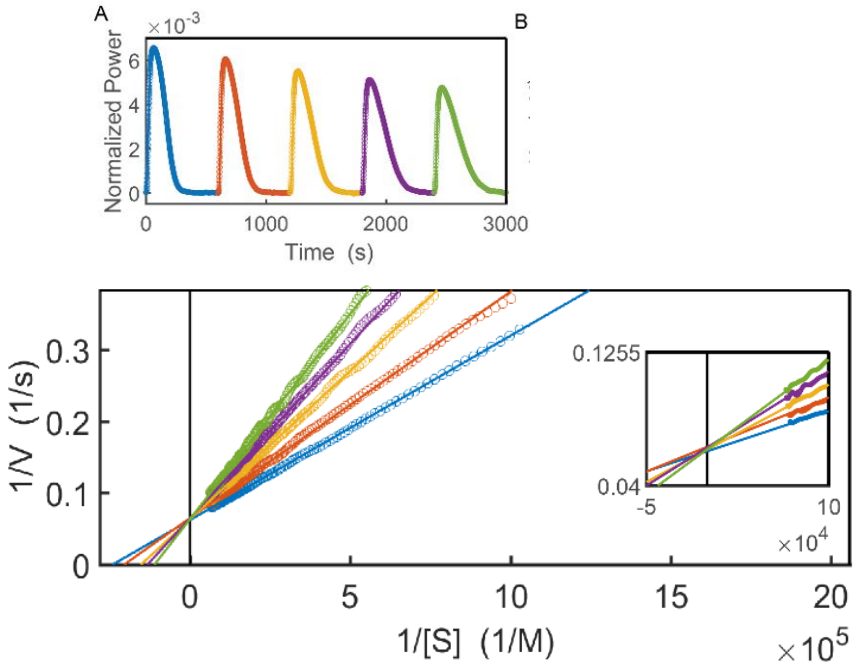


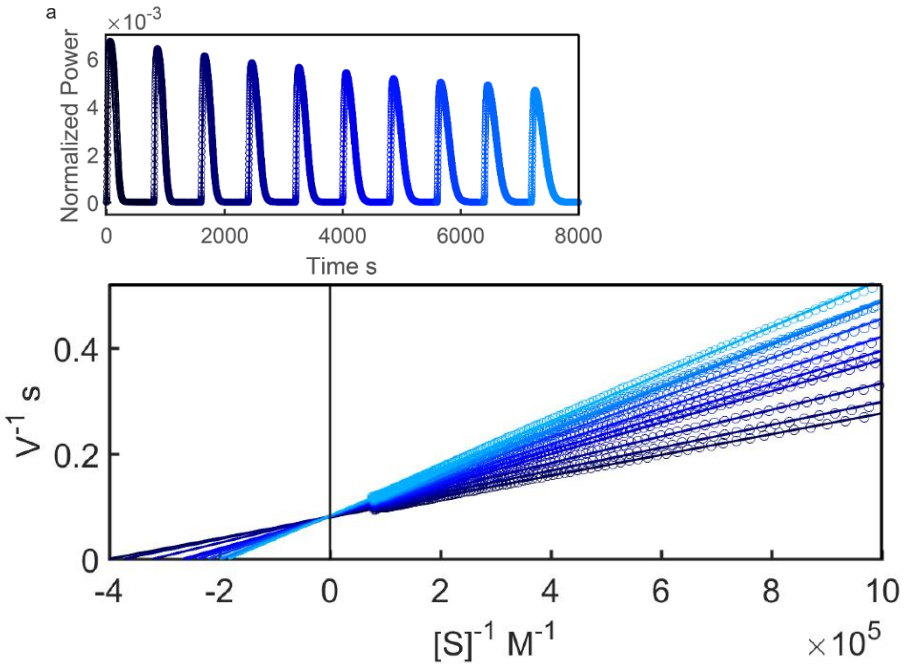


Strength & mode of inhibition (trypsin/benzamidine)

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

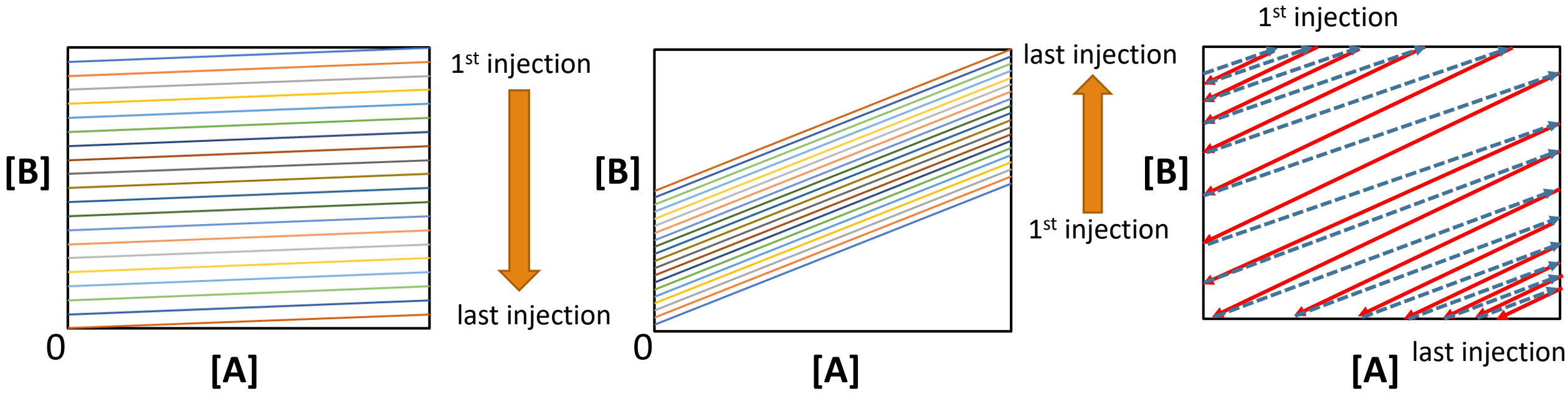
Both mode and affinity are determined rapidly in a single, hour-long experiment, a **5- to 10-fold savings in time and material**.





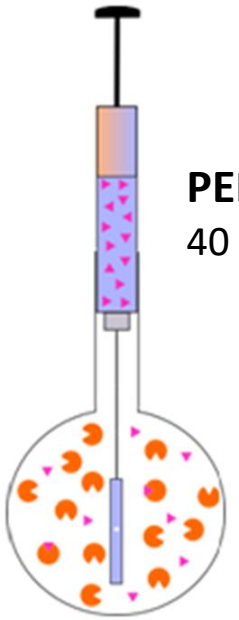
Bi-substrate enzymes (pyruvate kinase/PEP/ADP + Phe) 2D-ITC

$$\mathbf{A + B \longrightarrow P + Q}$$



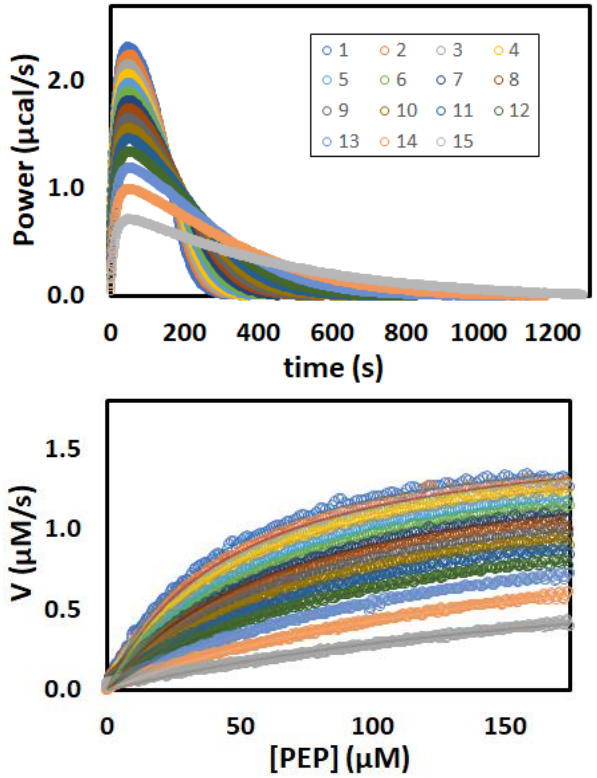
Pyruvate kinase/PEP/ADP + Phe

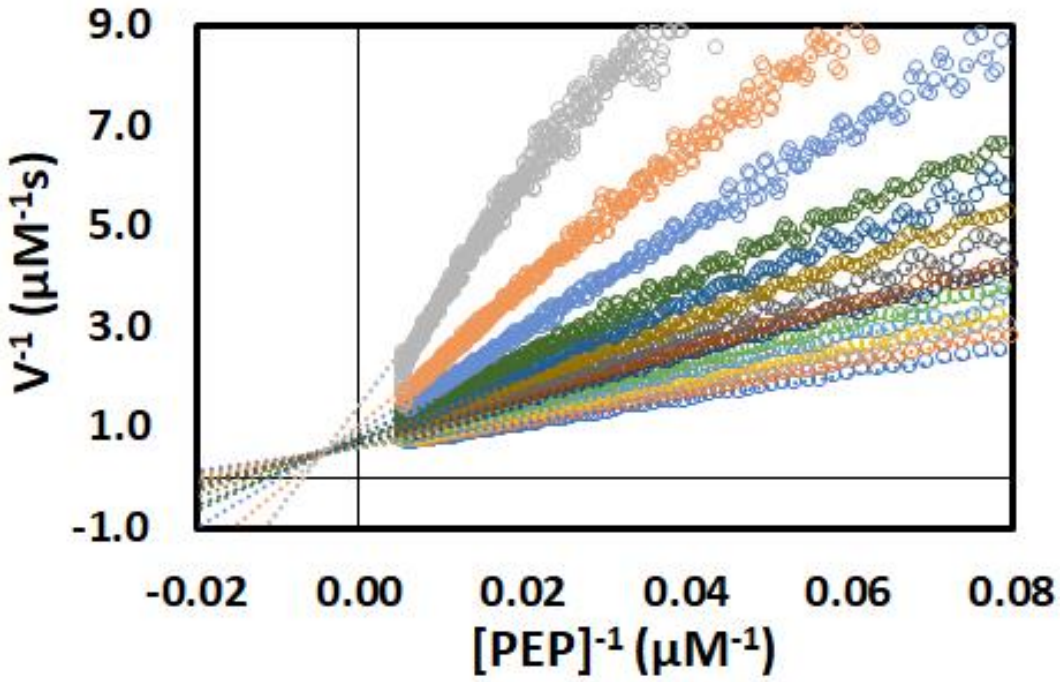
ADP in the cell is depleted with each injection.



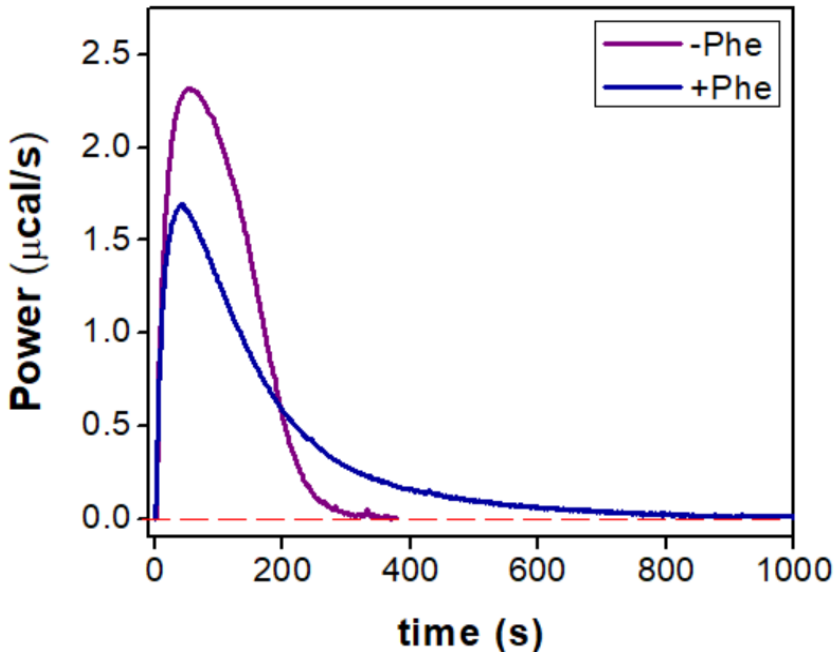
PEP
40 mM

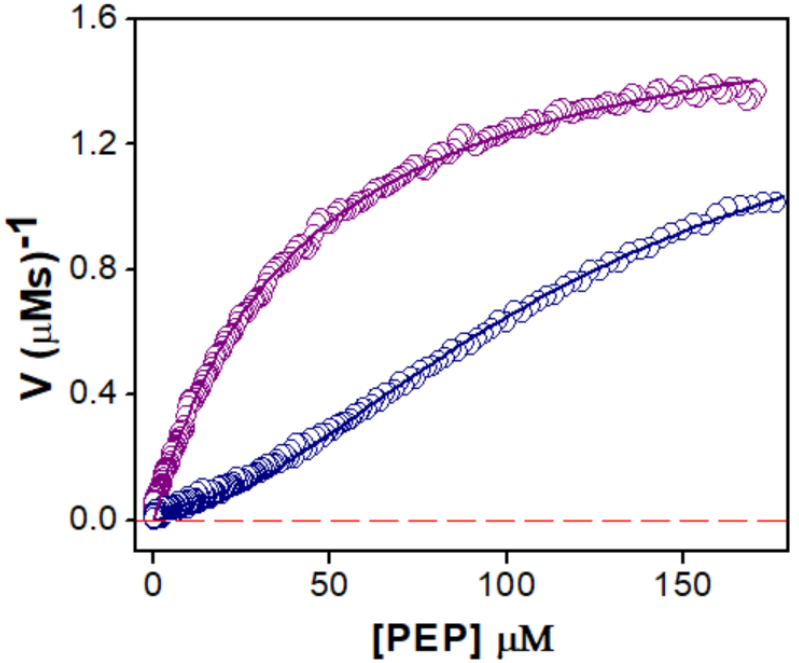
PK
8 nM
ADP
3.1 mM

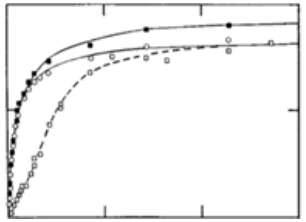




Pyruvate kinase/PEP/ADP + Phe





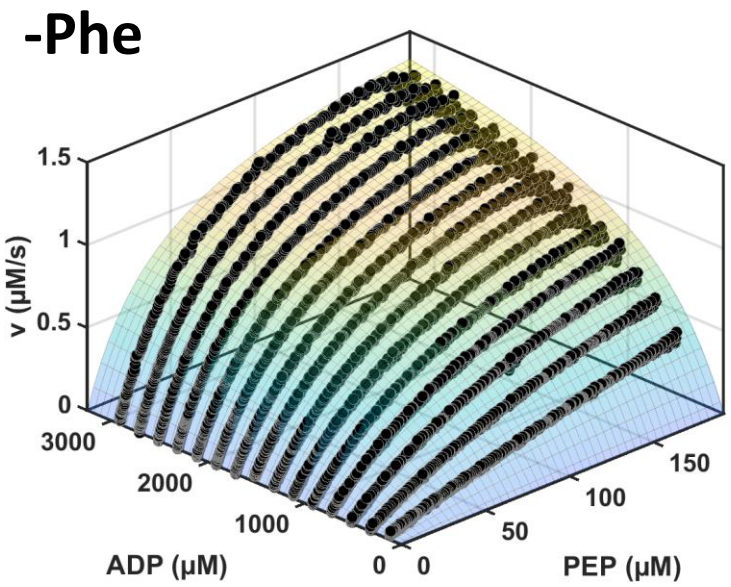


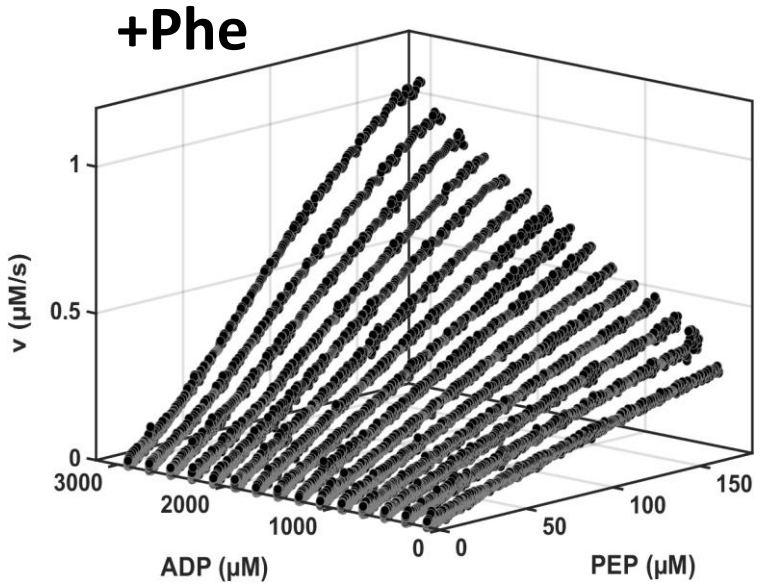
Biochemistry (2002) 41, 6897-6901

Two dimensional ITC

Michaelis Menten parameters (k_{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.

CIHR Canadian Institutes of Health Research

NSERC CRSNG

Canada Foundation for Innovation
Fondation canadienne pour l'innovation

Québec / Eastern Canada High Field NMR Facility

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