Electrophoretic Mobility Shift as a Detection Method for Inhibitors of Protein Kinases

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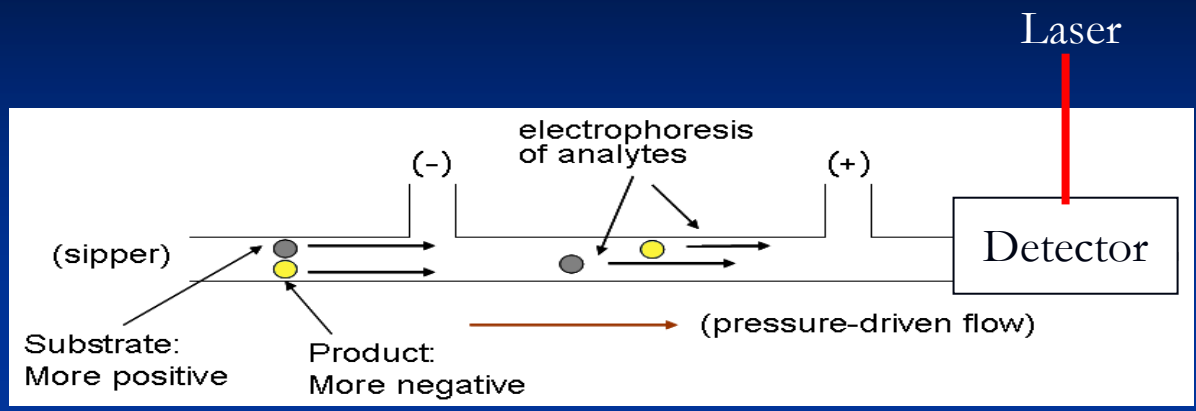
Introduction

- >500 protein kinases are present in the human genome
- Protein kinases regulate significant aspects of cell life through the phosphorylation of protein substrates
  - metabolism, cell cycle progression, cytoskeletal rearrangement, cell movement, apoptosis, differentiation
- Catalytic domain
  - Bisubstrate reaction
    - ATP/divalent cation binding
    - Protein/peptide substrate binding
    - Phosphate transfer from ATP to substrate
- Common catalytic mechanism among kinase family members leads to issues with inhibitor selectivity
- In-vitro kinase selectivity of drug candidates can be monitor with simple bioassay techniques

Filtration, the 'Golden Standard'

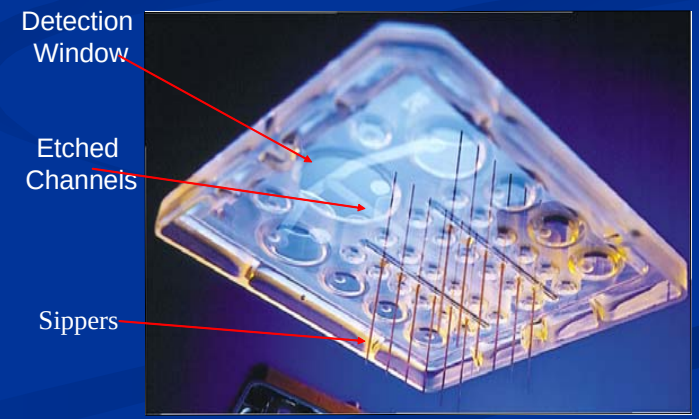
- Separates phosphorylated product from unphosphorylated substrate with little or no interference
- Discontinuous or end-point assay
  - Assumptions:
    1. Time point measurement is within the initial velocity phase of the reaction
    2. Conditions used to stop the reaction leads to an instantaneous and permanent halt of signal production
- Laborious and require the use of ^{33}P-γATP
- It is critical to restrict the assay time to the initial velocity phase of the reaction

Electrophoretic Mobility Shift Technology



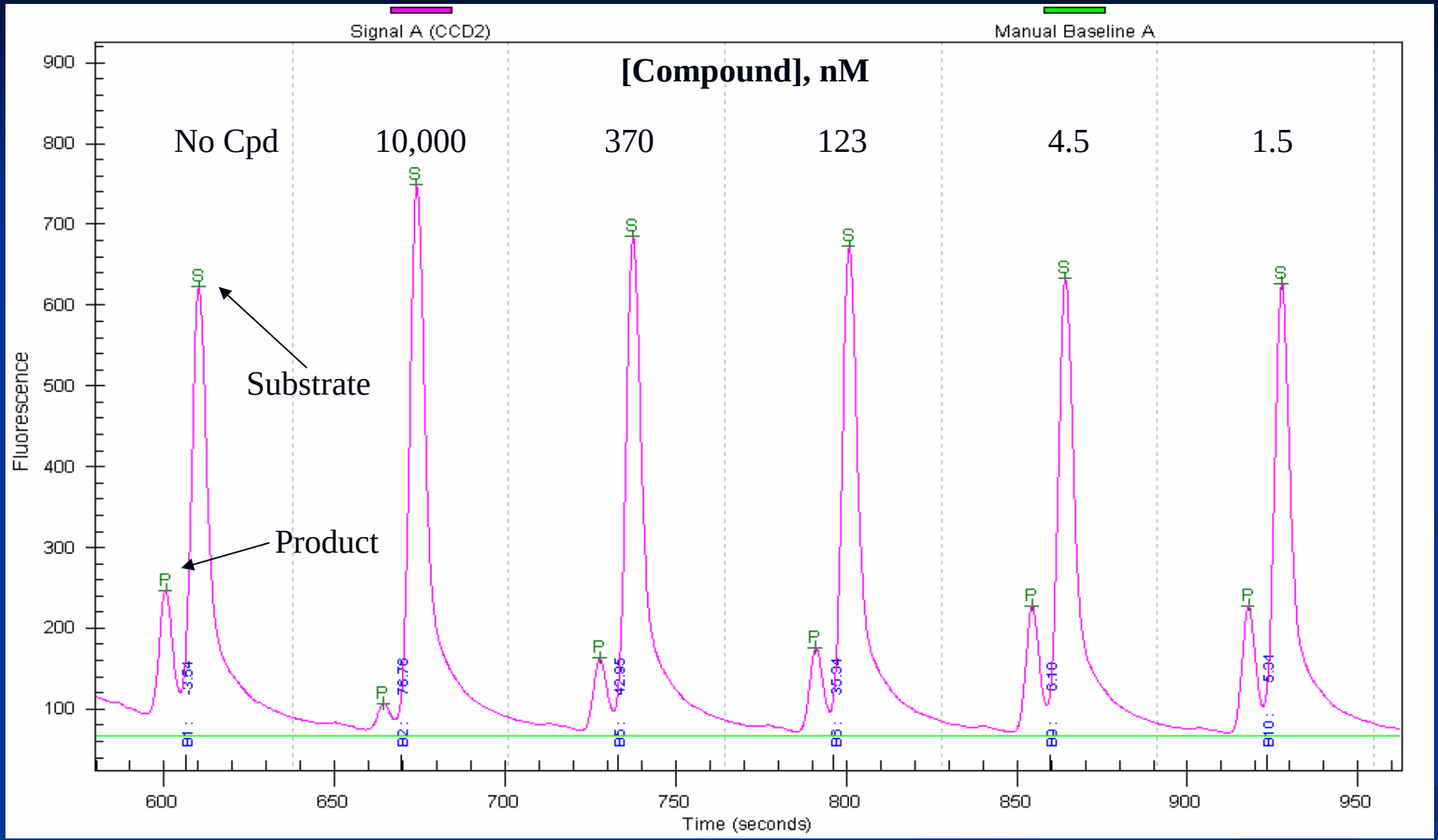
Electrophoretic separation of substrate and product

LabChip 3000



Microfluidic Chip

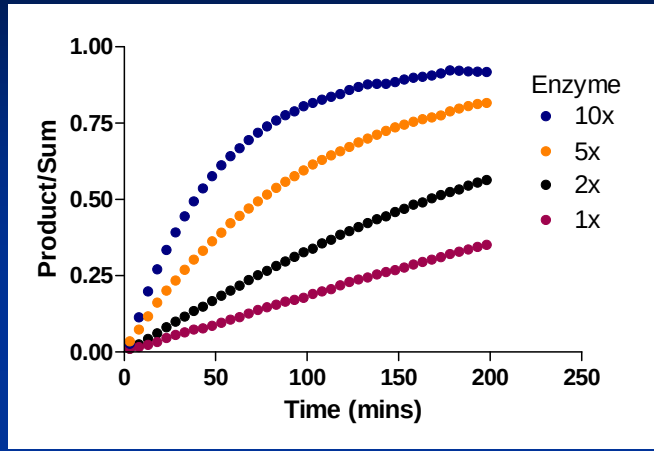
Detection of Protein Kinase Inhibition



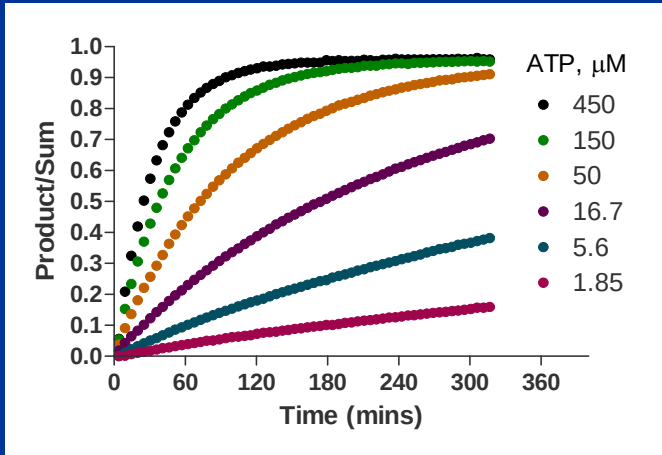
- Compound inhibition is determined by calculating P/(P+S) relative to control

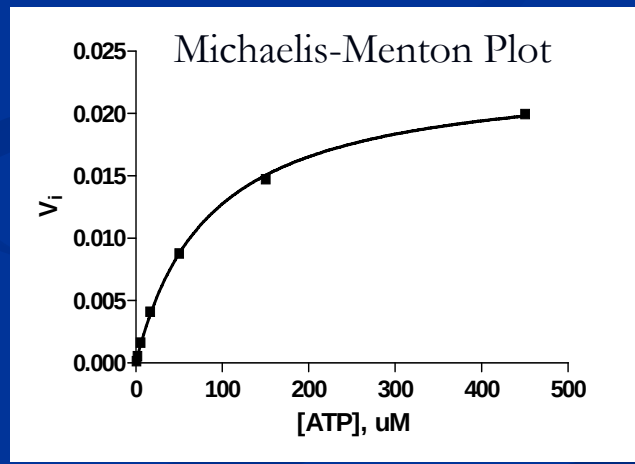
Mobility Shift Assay Development

Enzyme Titer



K_m^{app} for ATP





➢Continuous monitoring of the enzymatic reaction allows for accurate determination of kinetic constants under exact assay screening conditions

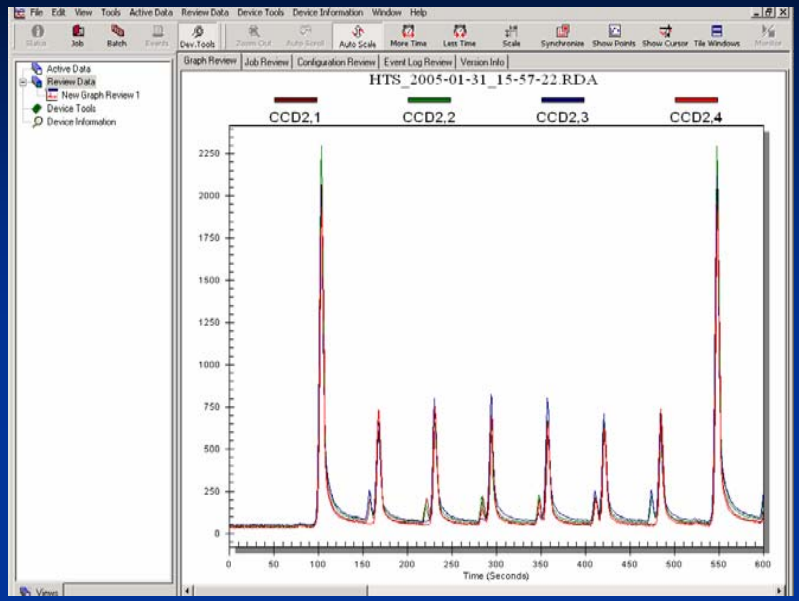
Correlation of ATP K_m^{app}

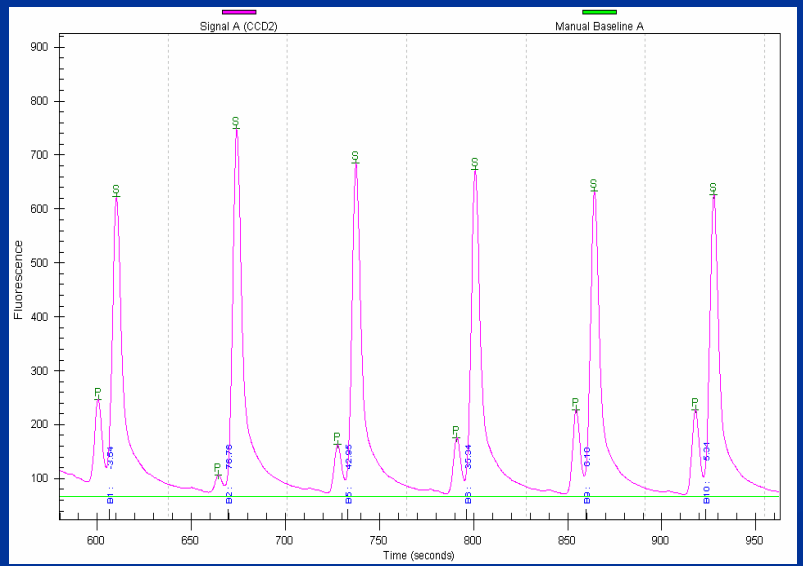
Enzyme Group	Filtration, Km (uM)	Mobility Shift, Km (uM)
TK	25	25
GMGC	33	84
AGC	25	25

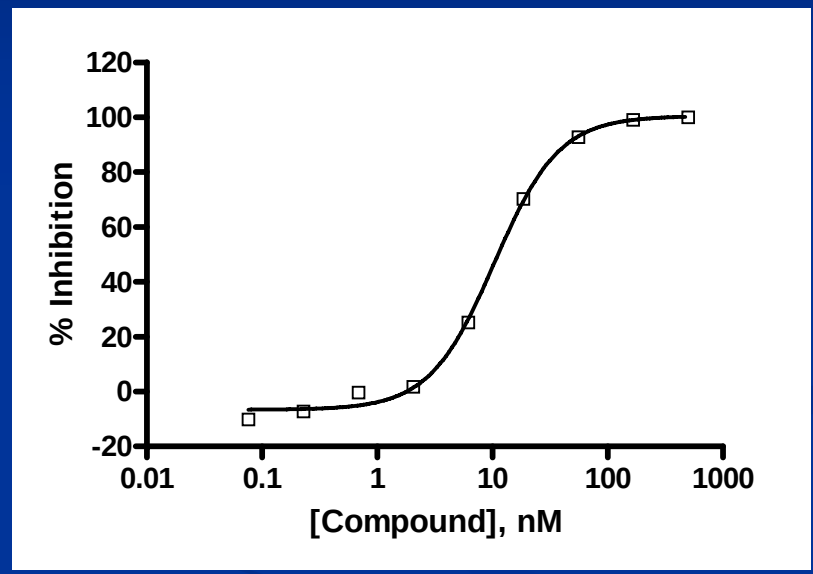
- Km values generated by mobility shift correlate with those from filtration

G. Manning, D. B. Whyte, R. Martinez, T. Hunter, S. Sudarsanam. The protein kinase complement of the human genome. Science298, 1912-1914 (2002).

Mobility Shift Data Analysis



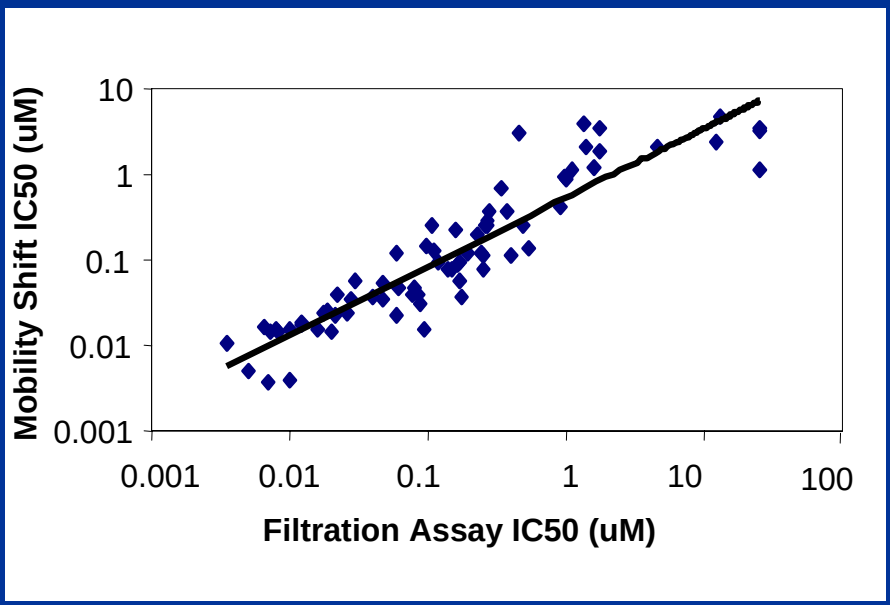




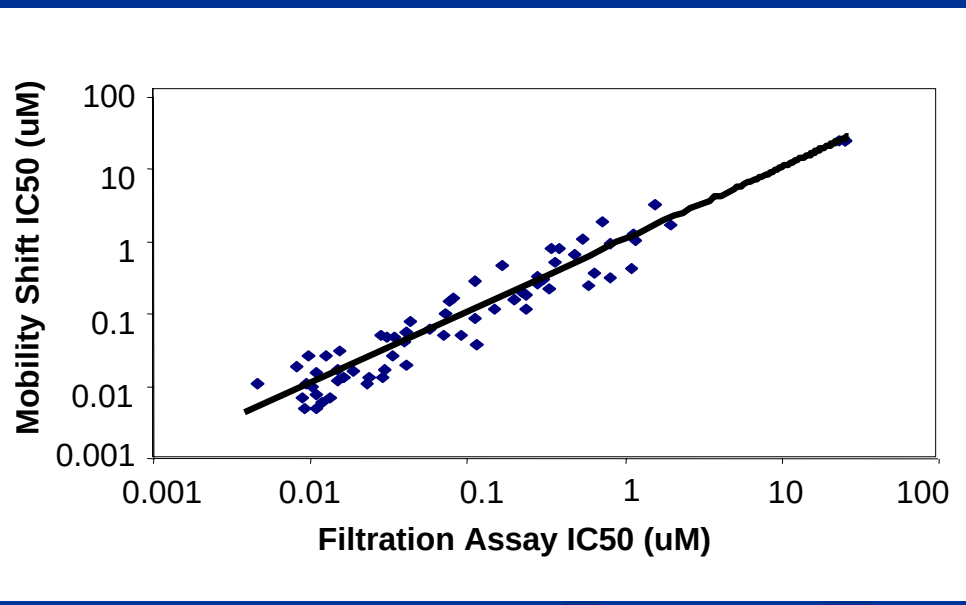
- Mobility shift data analysis is a multi-step process

Mobility Shift Assay Compound Validation

Tyr Kinase Inhibition

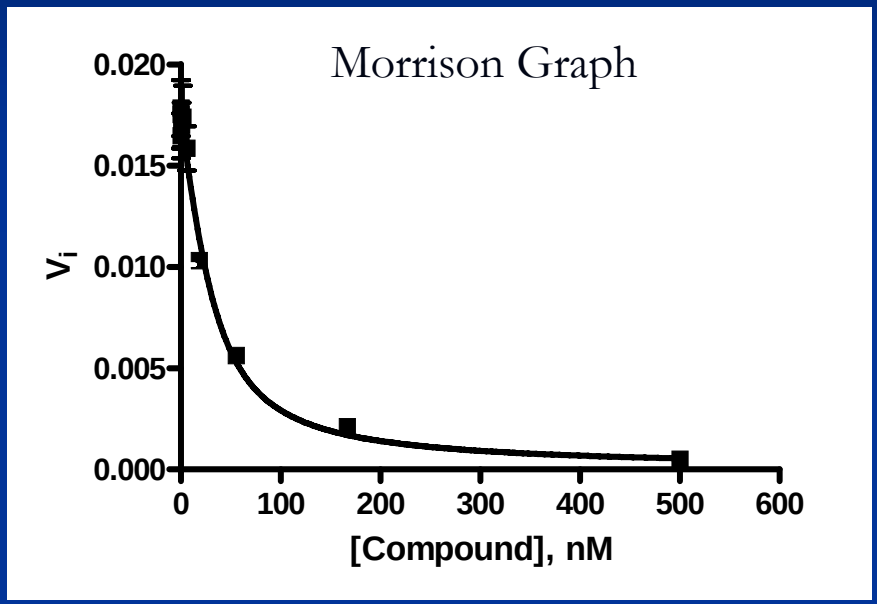


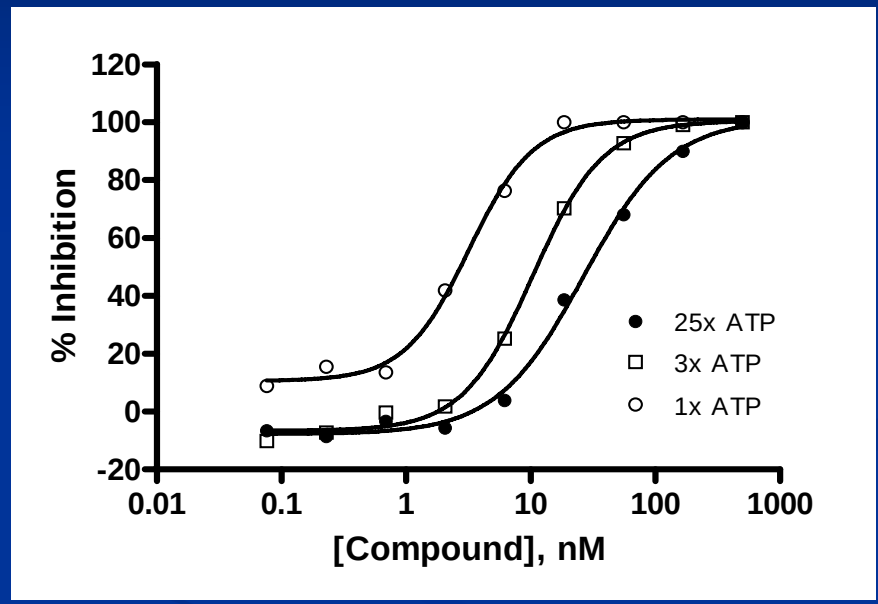
Ser/Thr Kinase Inhibition



- Correlation between filtration and mobility shift assays is observed for both inhibitor types

Compound Kinetic Characterization using the Mobility Shift Assay





➢The evaluation of lead compounds using the mobility shift assay allows for steady state determination of compound potency and mechanism of action

Summary

- Filtration is a radioactive, laborious, and discontinuous assay method requiring enzyme quenching prior to signal detection
- Electorphoretic mobility shift allows for enzymatic characterization and lead compound evaluation using initial velocity measurements
- The mobility shift assay is an ideal method for measuring the initial enzymatic rate and makes subsequent analysis of reaction mechanism and inhibition modality uncomplicated and reproducible