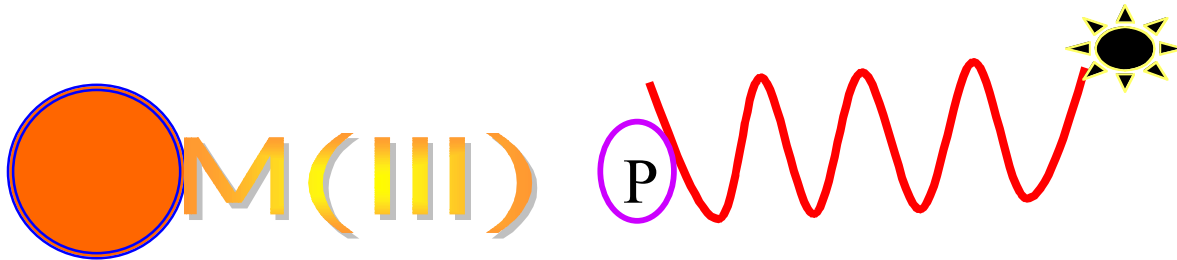


IMAP™: A New Assay Technology for HTS of Phosphodiesterases, Kinases and Phosphatases

Wei Huang, Yang Zhang and Rich Sportsman

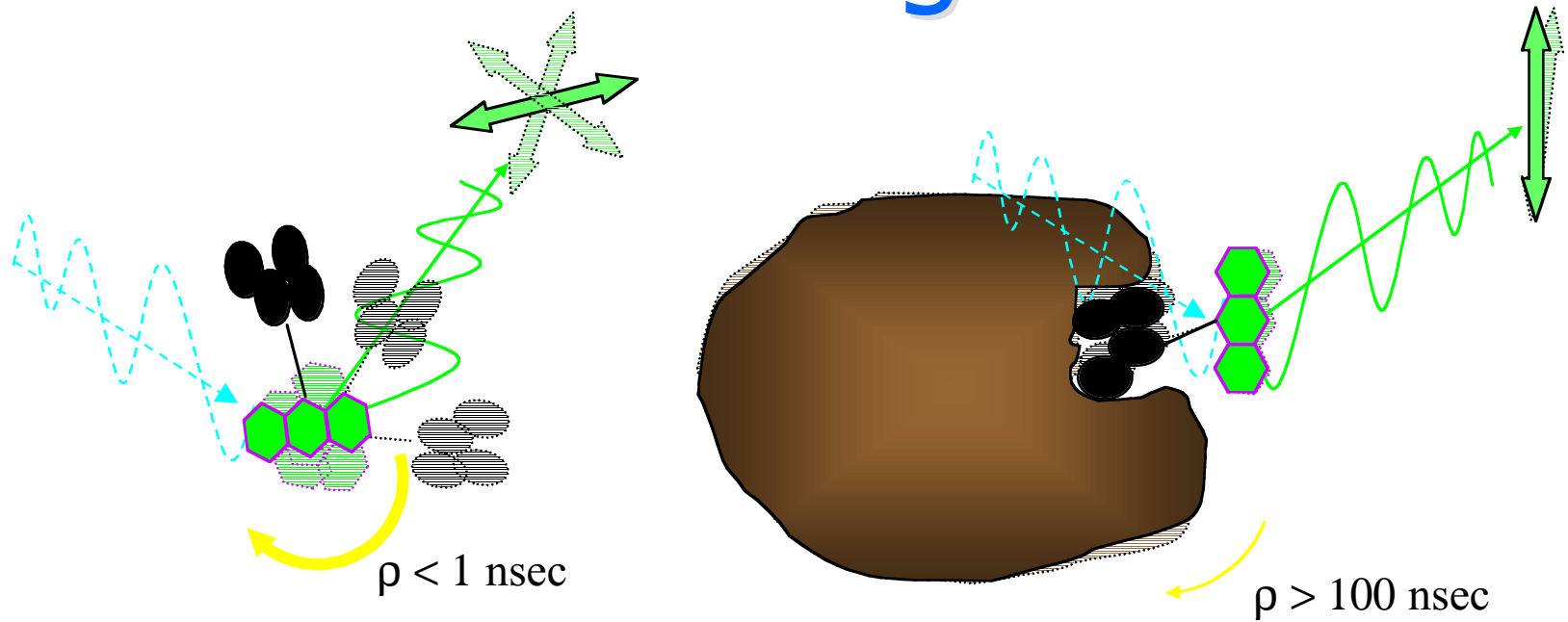
IMAP™ Technology

(Immobilized Metal Assay for Phospho-chemicals)



- Proprietary Binding materials -- nanoparticles with metal cations on the surfaces, have been shown to bind specifically to phosphopeptides and phosphoproteins.
- Interference: phosphate, ATP.
- Patent pending

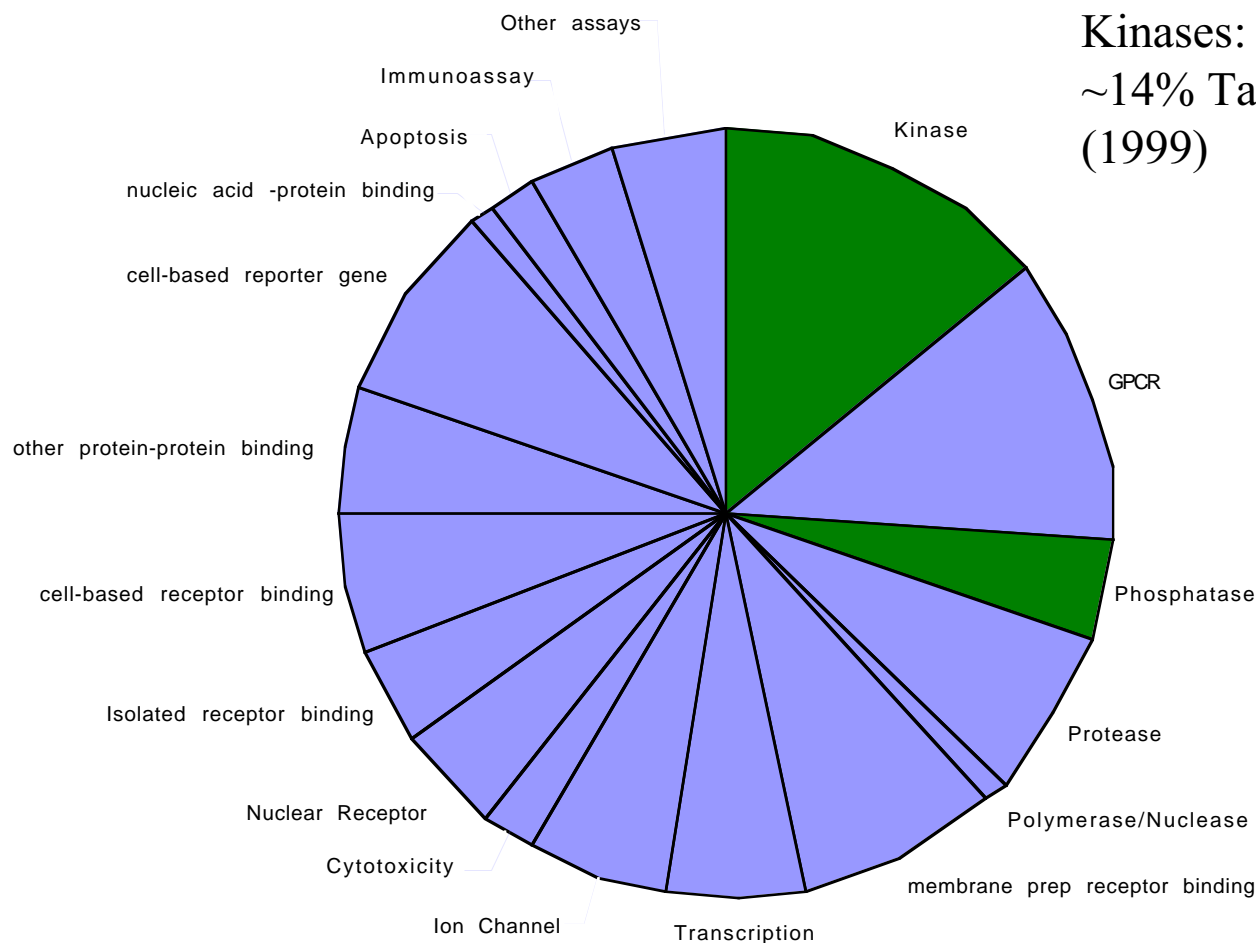
Fluorescence Polarization and Binding



“Free” fluorescent ligand -
rapid rotation, *depolarized* emission

“Bound” fluorescent ligand -
slow rotation, *polarized* emission

Applications of IMAP™ Technology

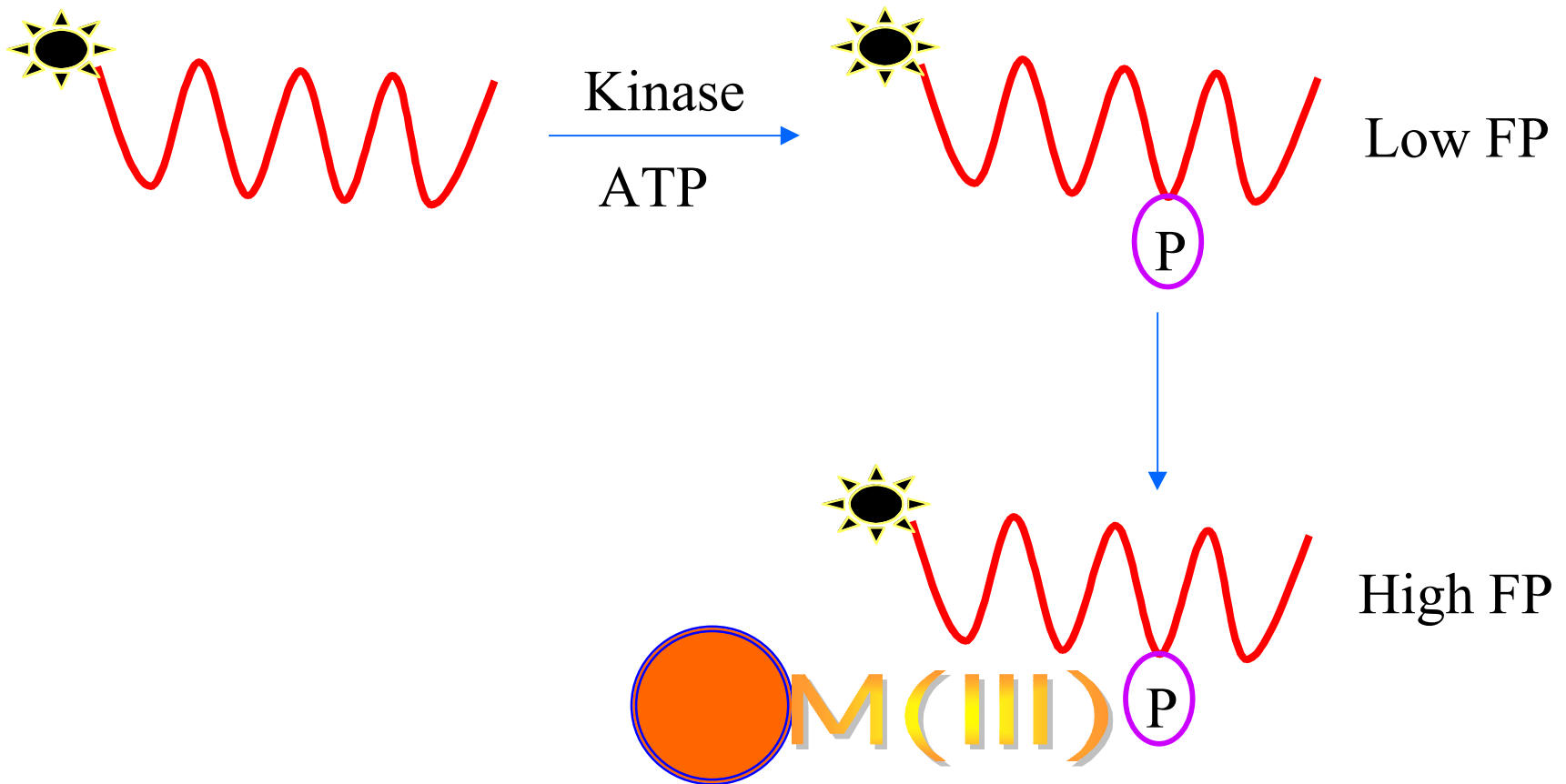


Kinases: ~1000 genes
~14% Target for HTS
(1999)

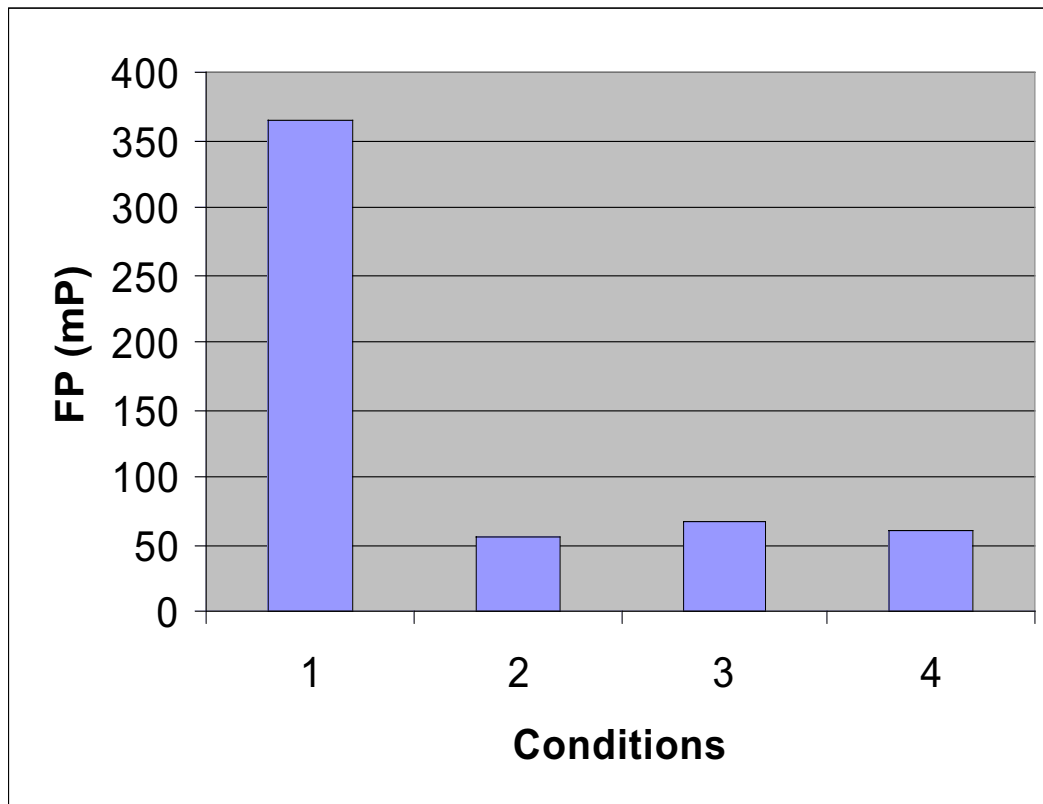
Development of Kinase Assay

- Antibody based assays:
 - Tyrosine kinase – generic assay available
 - Serine/Threonine – No generic antibody available
- ☹ Usually takes 3-6 months to develop Ab
- Radioactive assays:
 - ^{32}P , ^{33}P -labeled ATP
 - Heterogeneous format: filter plate
 - Homogeneous format: flash plate (SPA)
- ☹ Generic but difficult

IMAP™ for Generic Kinase Assay

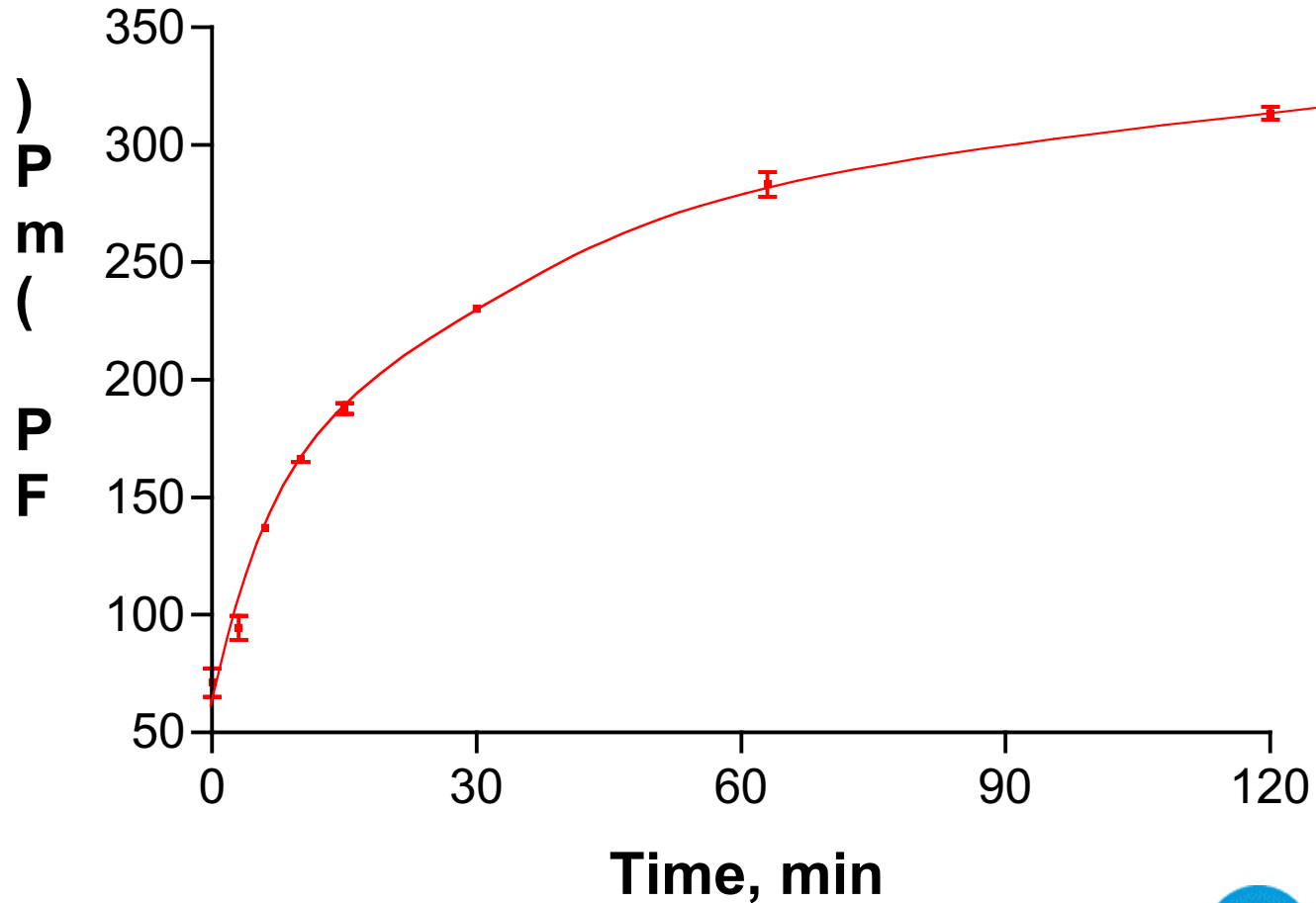


End-Point Study

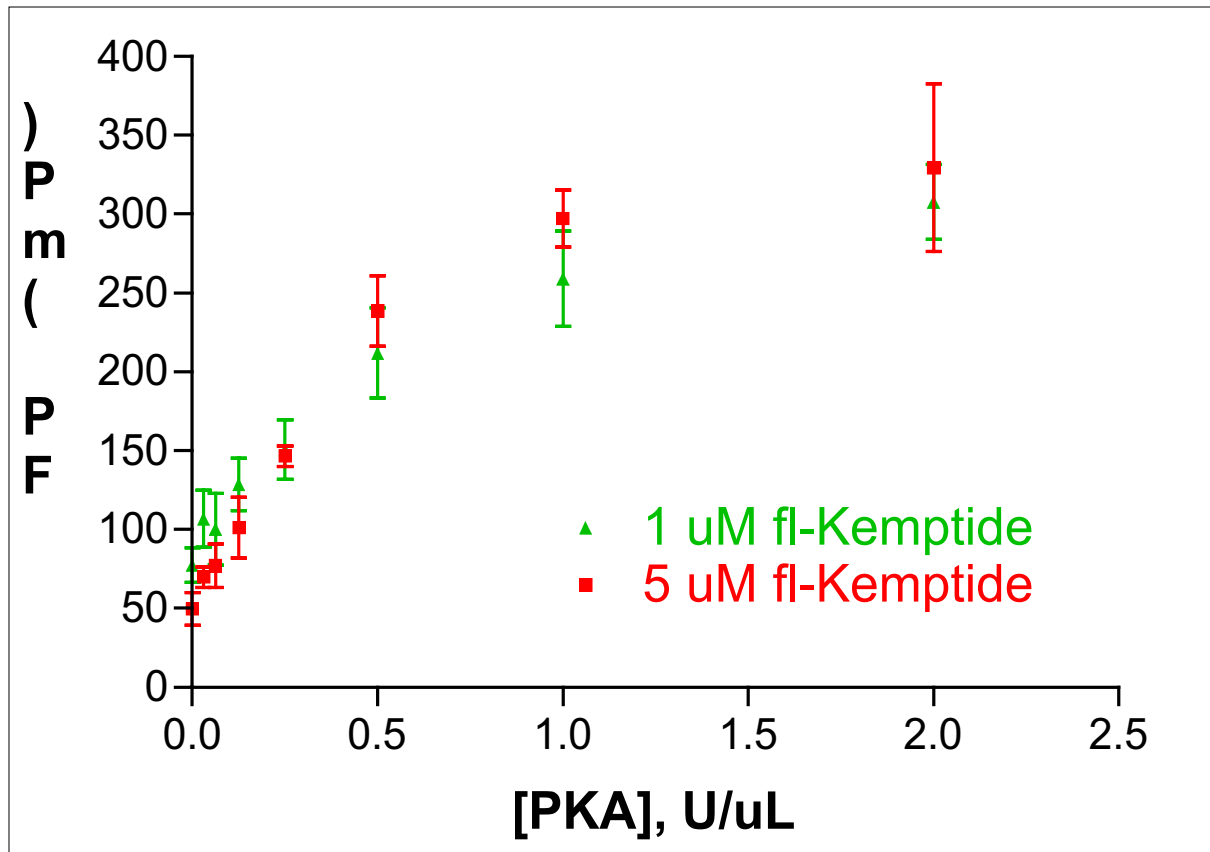


1) reaction with enzyme, assay with IMAP Binding Material; 2) reaction with enzyme, no IMAP Binding Material; 3) no enzyme control, with IMAP Binding Material; 4) no enzyme control, no IMAP Binding Material.

Time-Course Study

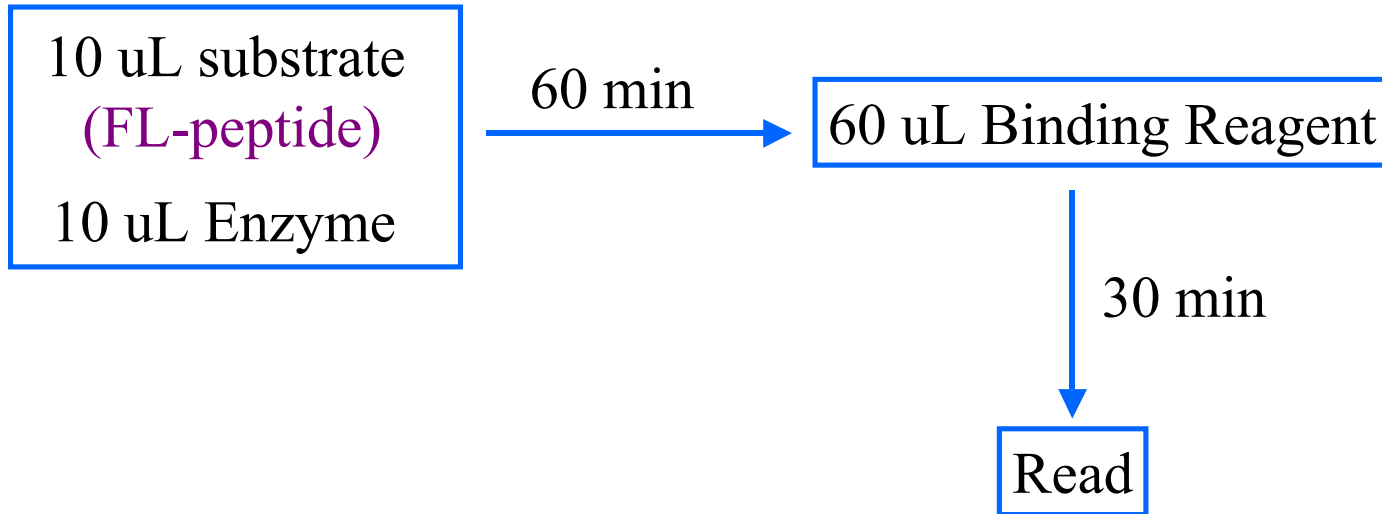


PKA Activity Titration

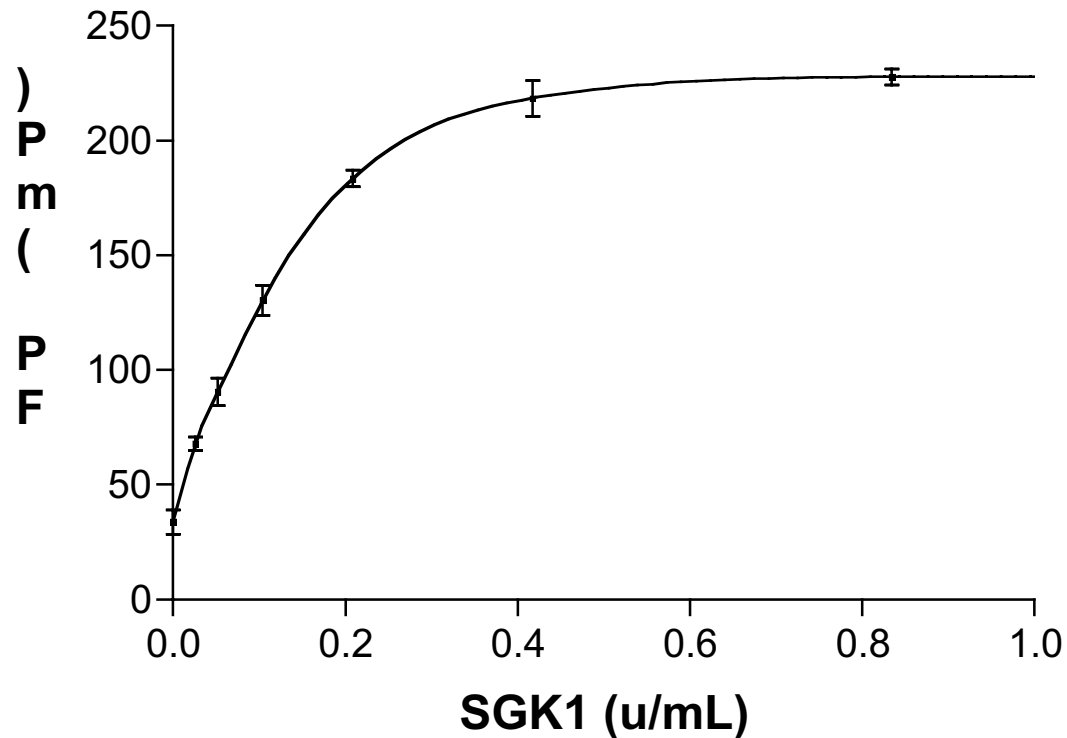


IMAP™ Kinase Assay

384-well plate Assay

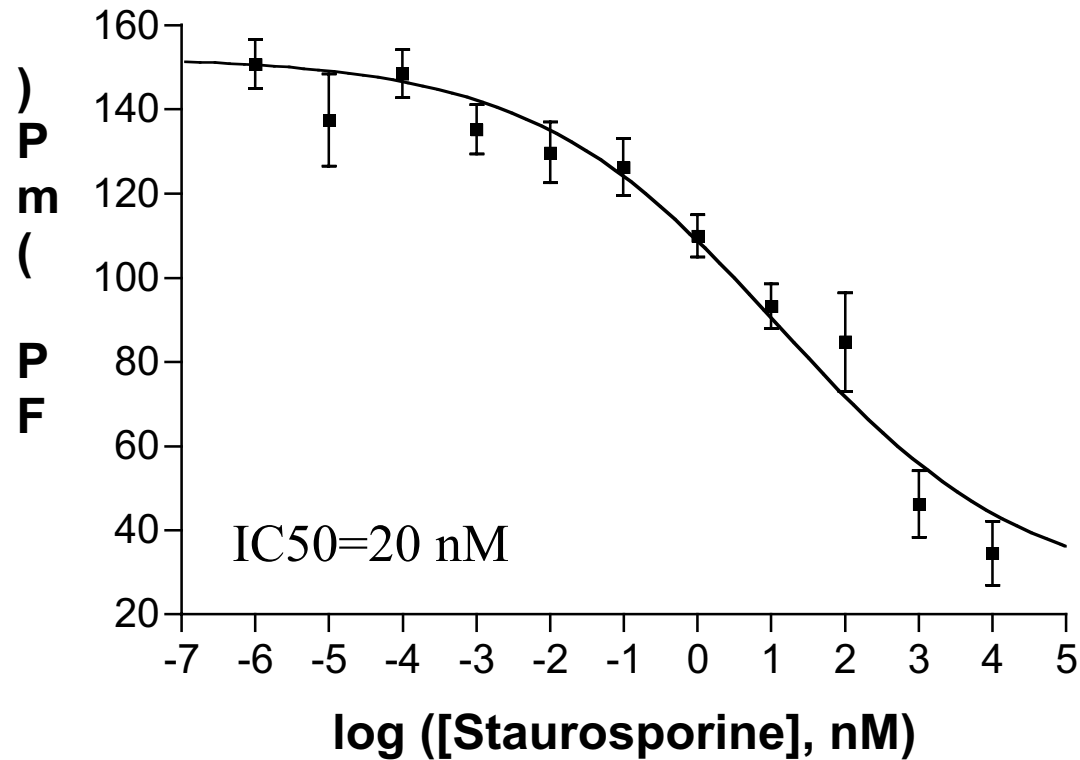


SGK Activity Titration



(10 uM ATP, 1 uM fl-Crosstide, RT, 60 min)

IC50 for SGK

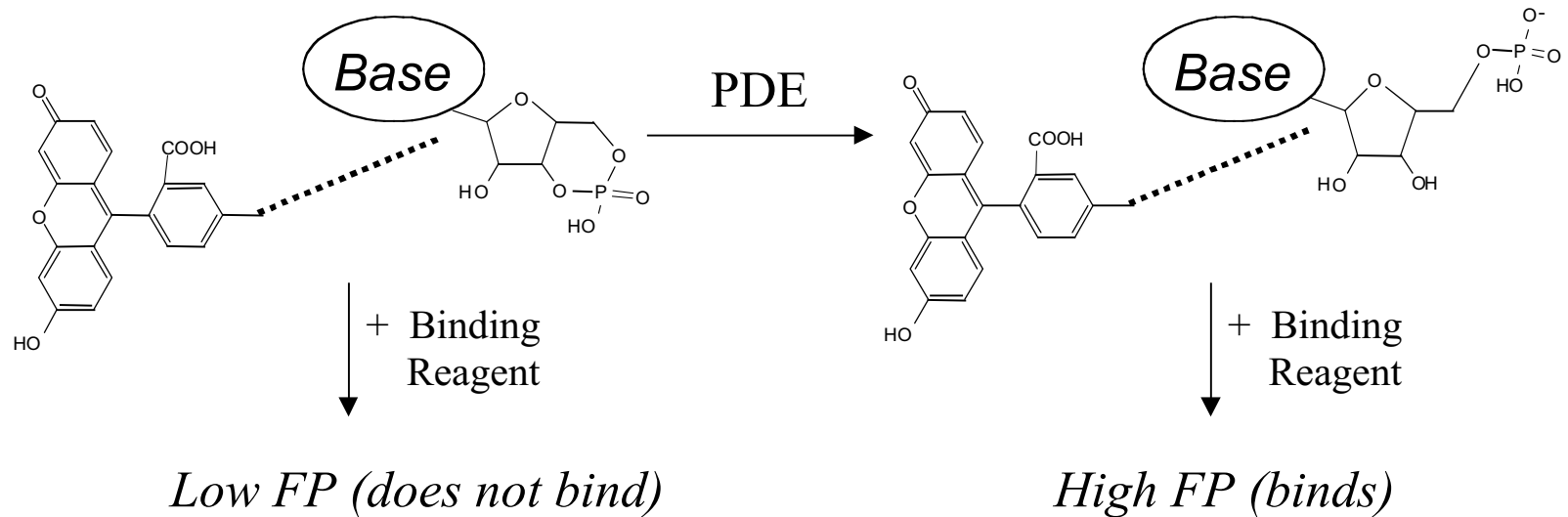


(0.27 u/mL SGK, 10 uM ATP, 1 uM fl-Crosstide, RT, 60 min.)

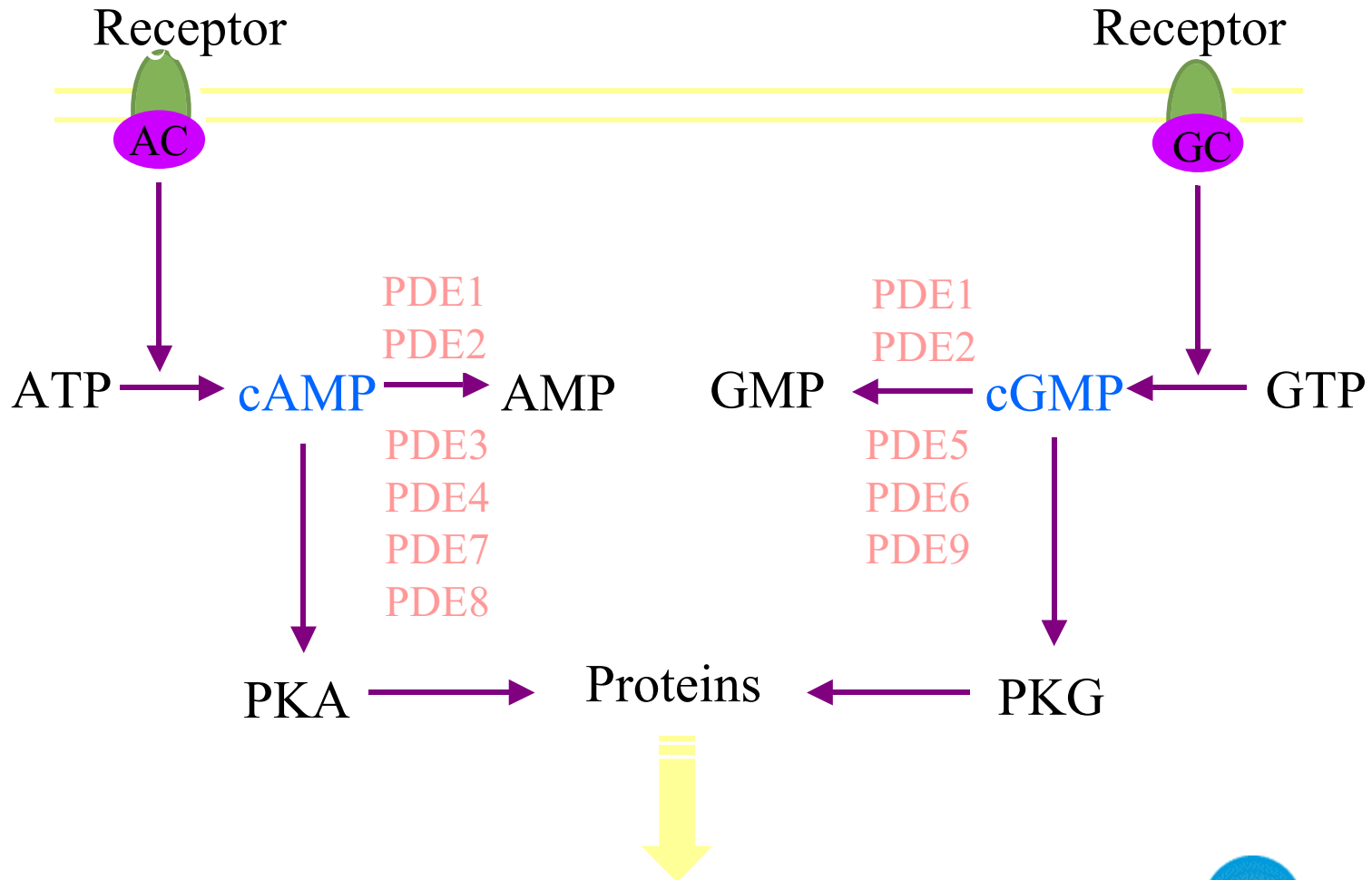
Summary of IMAP™ Kinase Assay

- Advantages:
 - Generic, not antibody-based
 - Homogeneous, FP detection.
 - Reproducible, cost-effective, robust
- Disadvantages:
 - Interference from ATP and phosphate need to be controlled.

IMAP™ Assay for Phosphodiesterase



Phosphodiesterase (PDE)

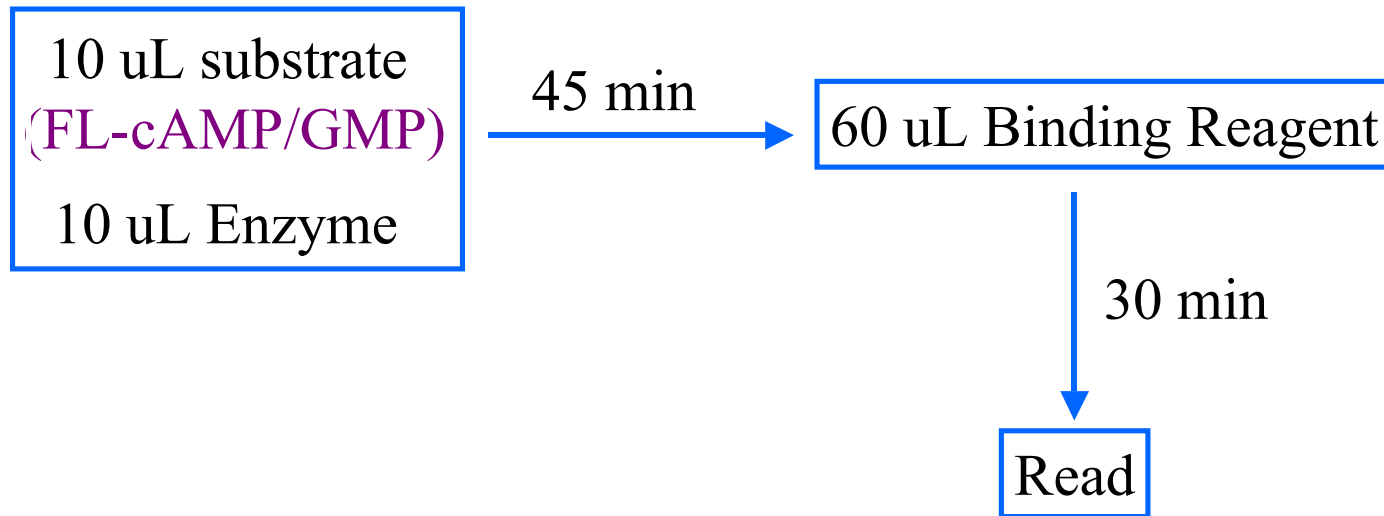


PDE as Drug Target

- More than 30 human PDE genes identified.
- Classified into 11 classes.
- First commercialized PDE inhibitor (PDE5)
- Viagra.
- A number of drugs in clinical trials for asthma (PDE4).
- Current HTS assay: SPA (Amersham PB).

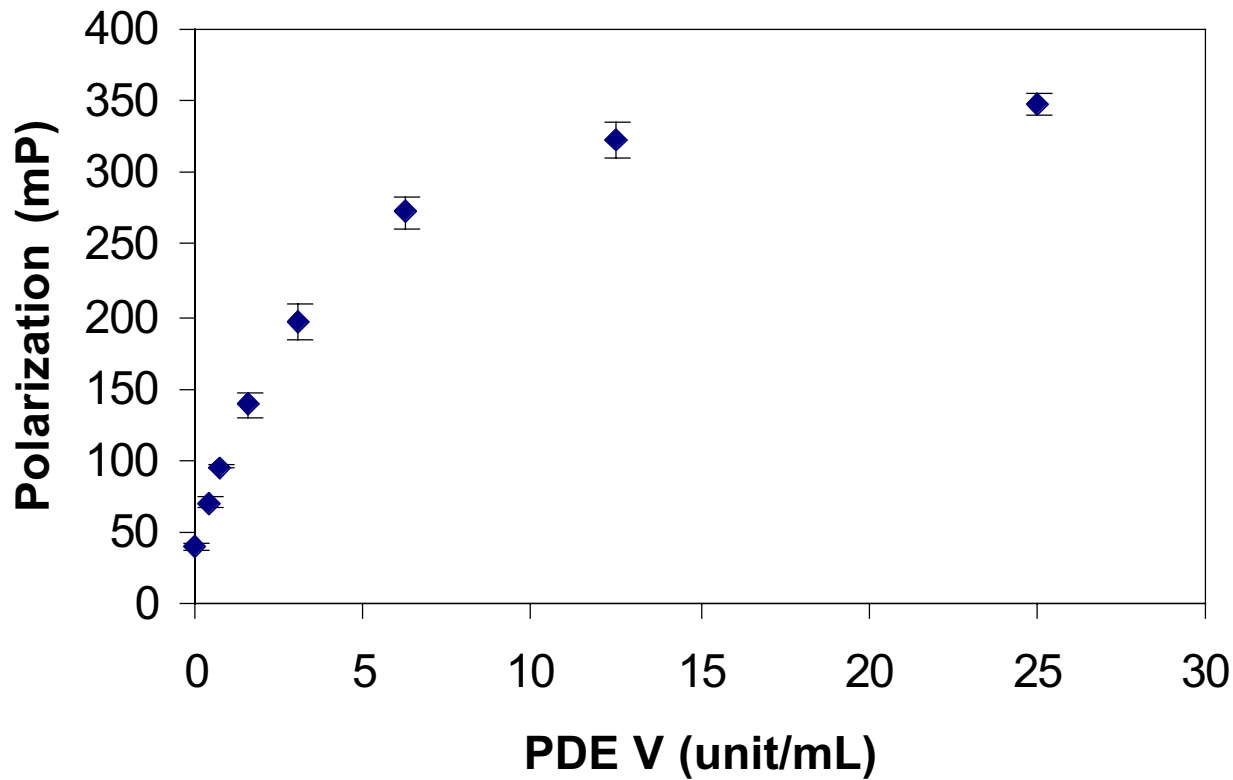
IMAP™ Phosphodiesterase Assay

384-well plate Assay

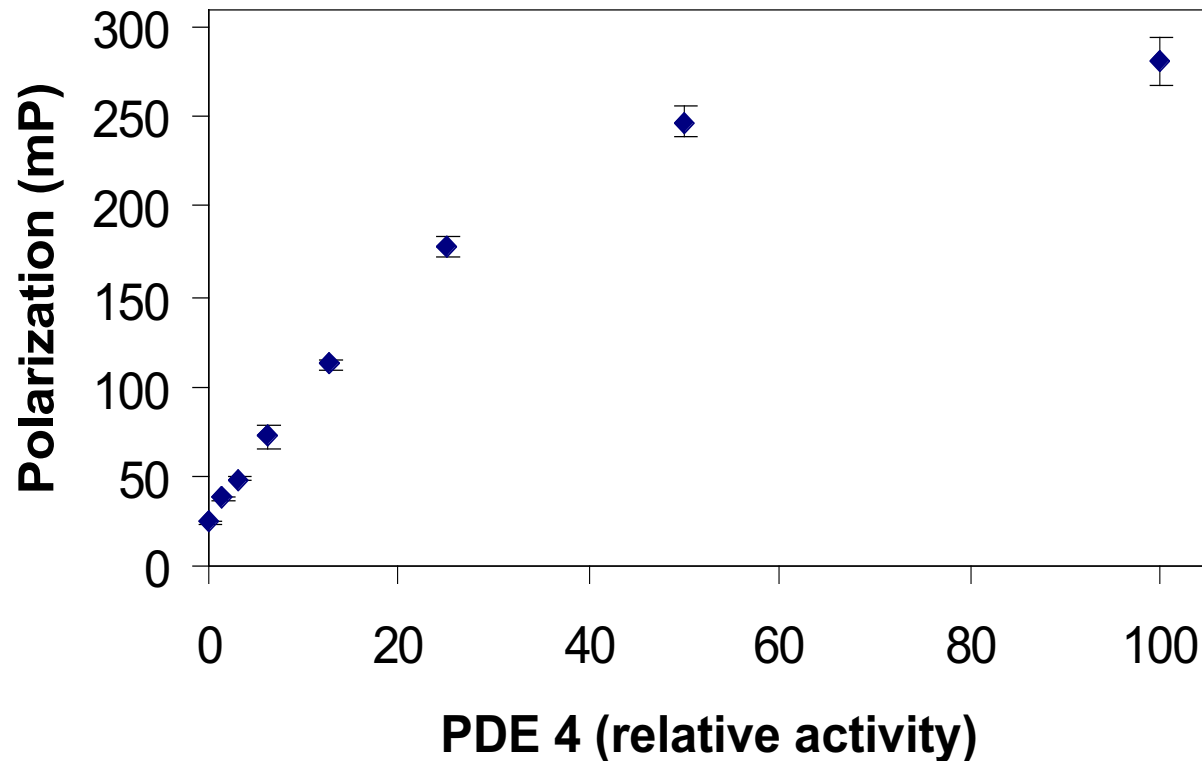


The Assay tested on PDE 4, PDE 5, and PDE 1

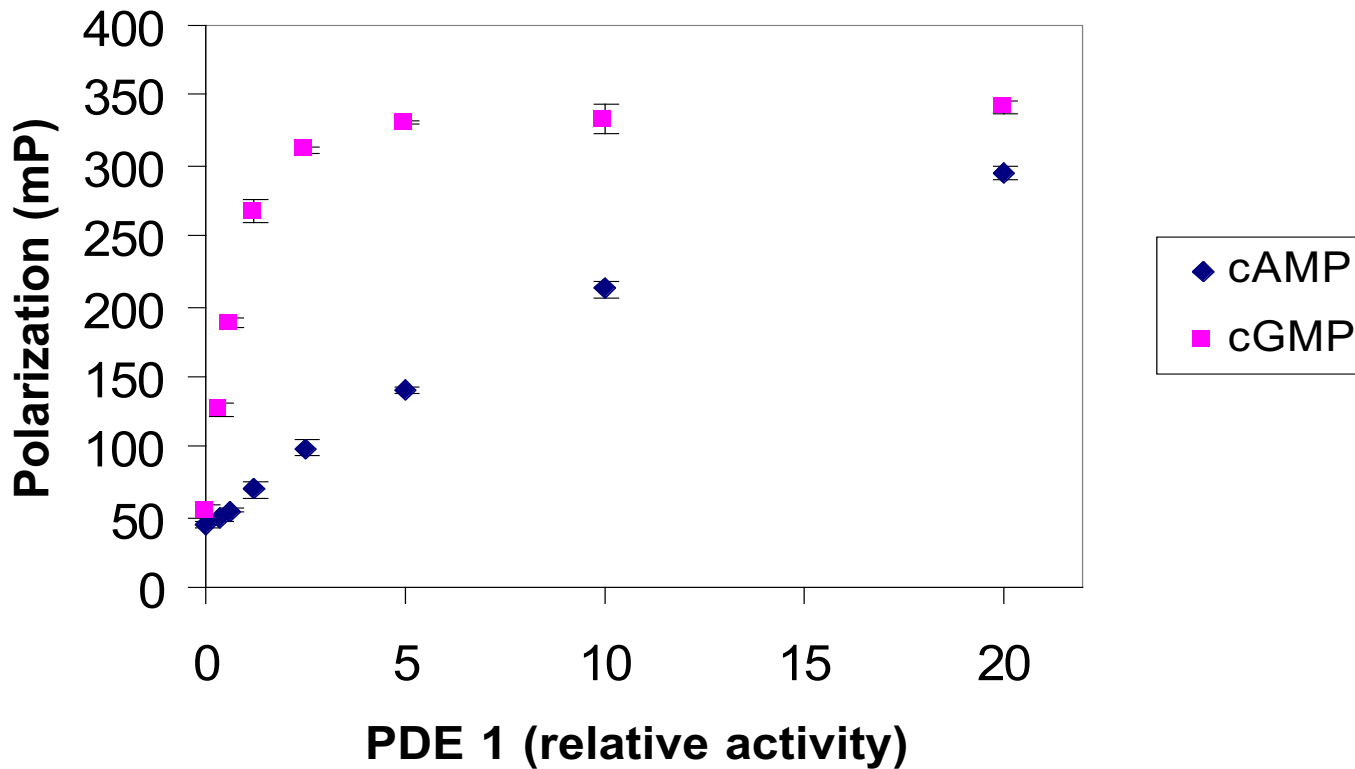
IMAP™ Phosphodiesterase Assay (PDE 5)



IMAP™ Phosphodiesterase Assay (PDE 4)

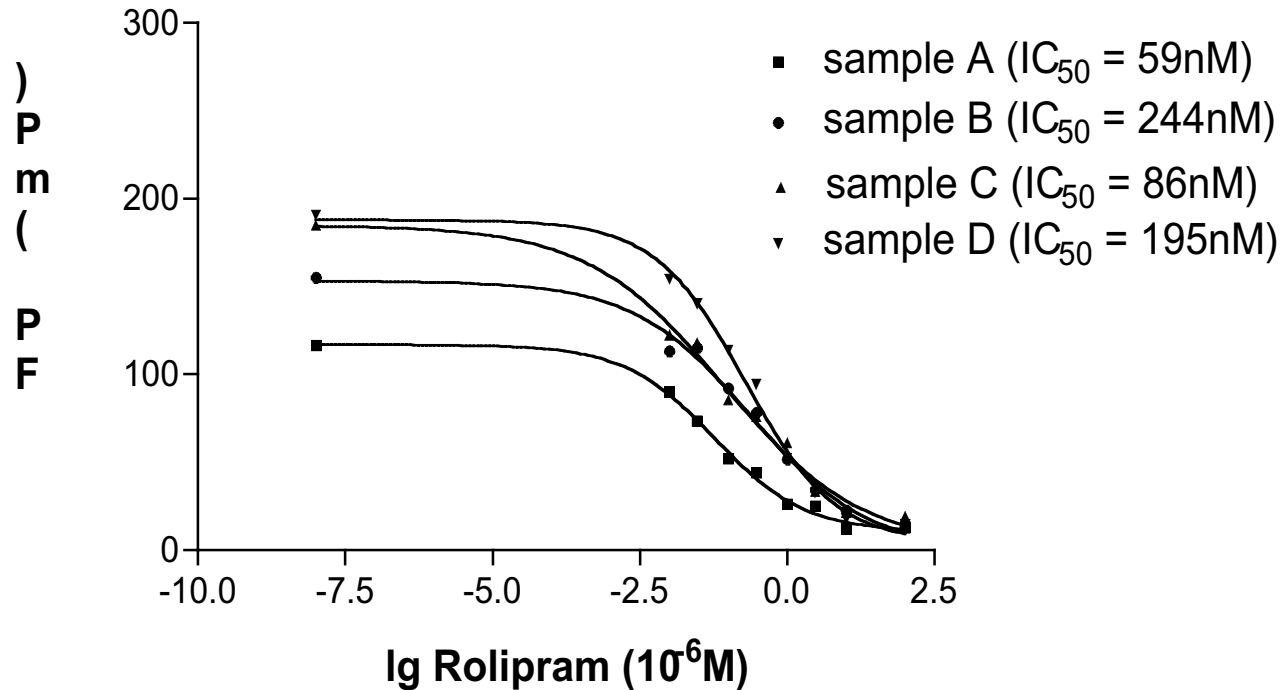


IMAP™ Phosphodiesterase Assay (PDE 1)



IC50 Testing

**IC₅₀-values for XB4A refolded
from inclusion bodies**



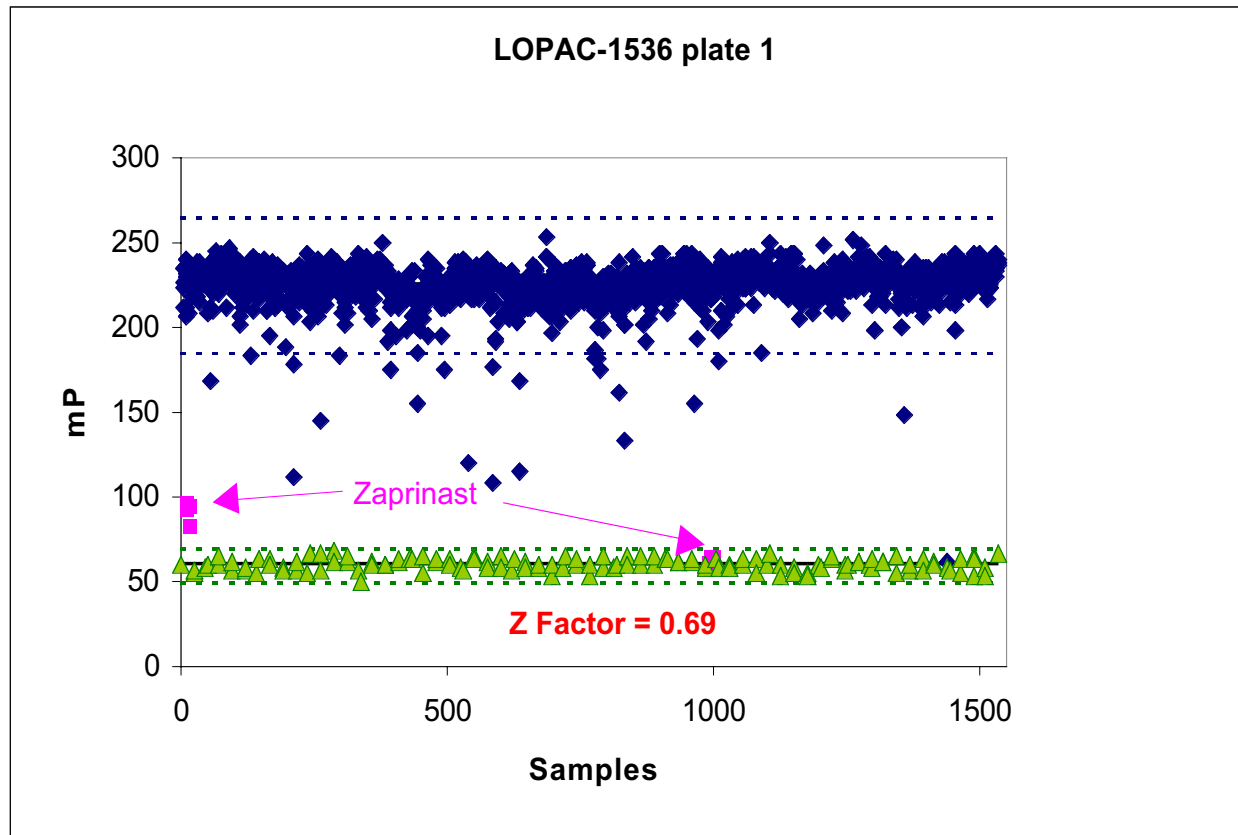
Courtesy Drs. M. Conti, W. Richter, Stanford University

ScreenStation™ for Homogeneous Assays



Screening on ScreenStation™

1536-well PDE Assay on ScreenStation



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