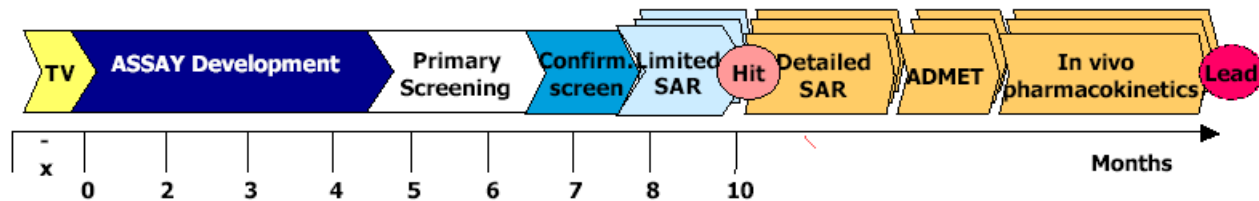


# **Two simple and generic antibody-independent kinase assays: Comparison of a bioluminescent and a microfluidic assay format**

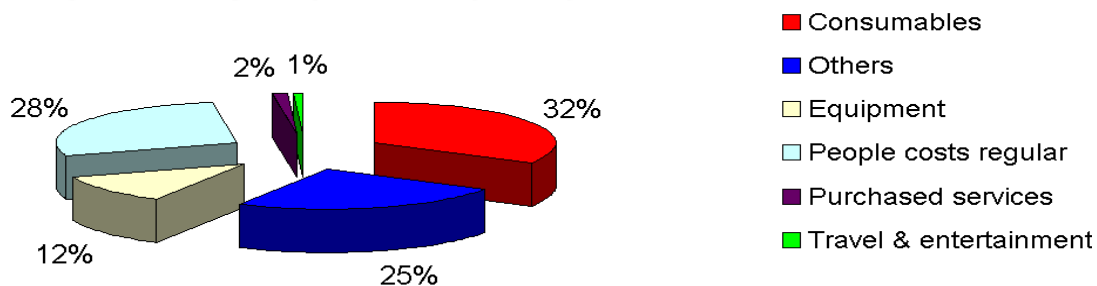
**Antje Pommereau, Everard Pap, Aimo Kannt**

# Challenges in kinase assay development

- reduce AD cycle times



- reduce cost of consumables

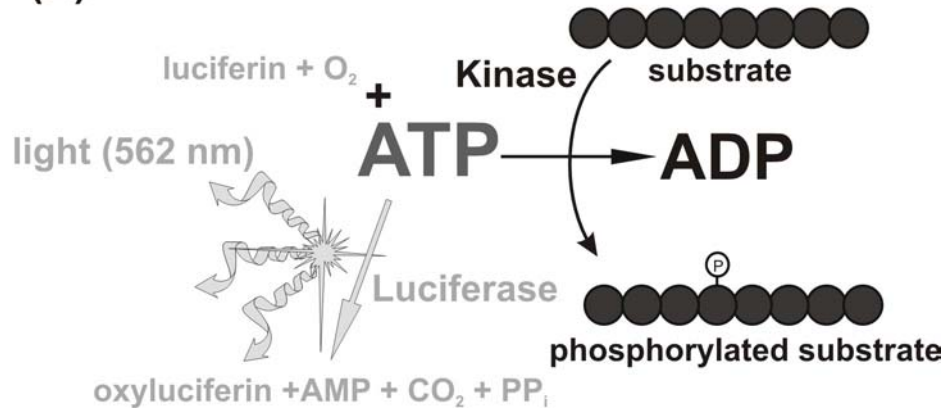


- selectivity: generic assays with high data quality

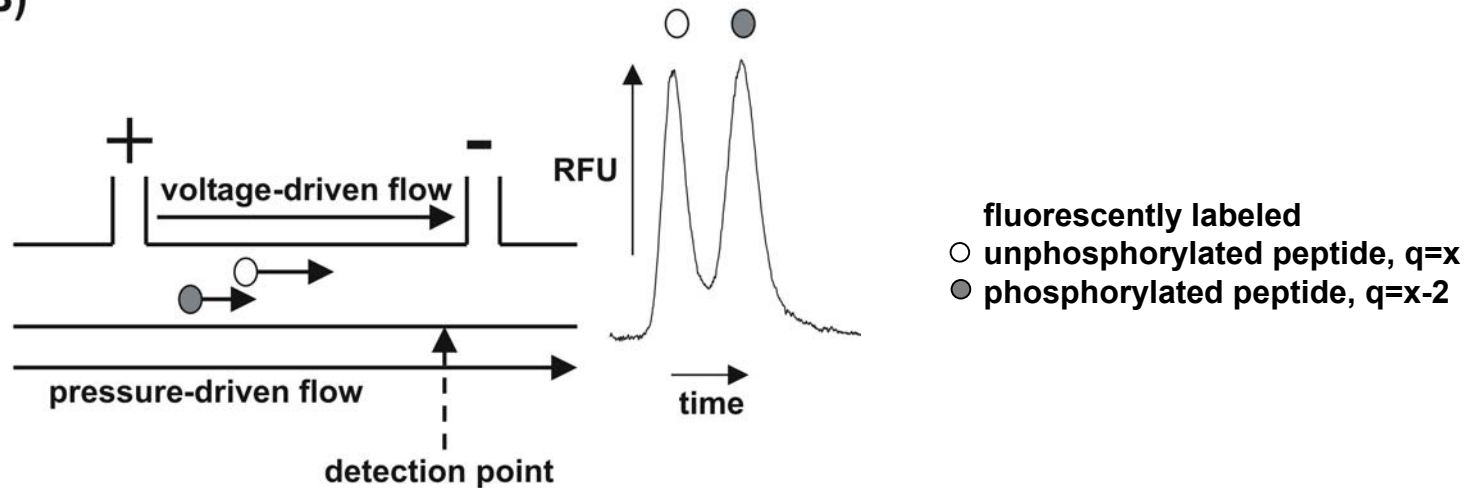
IC50 (µM)	kinase A	kinase B	Kinase C	kinase D
cpd. 1	>100	0.2	15.3	>100
cpd. 2	2.7	1.8	>100	>100
cpd. 3	>100	>100	2.3	>100

# Assay principles

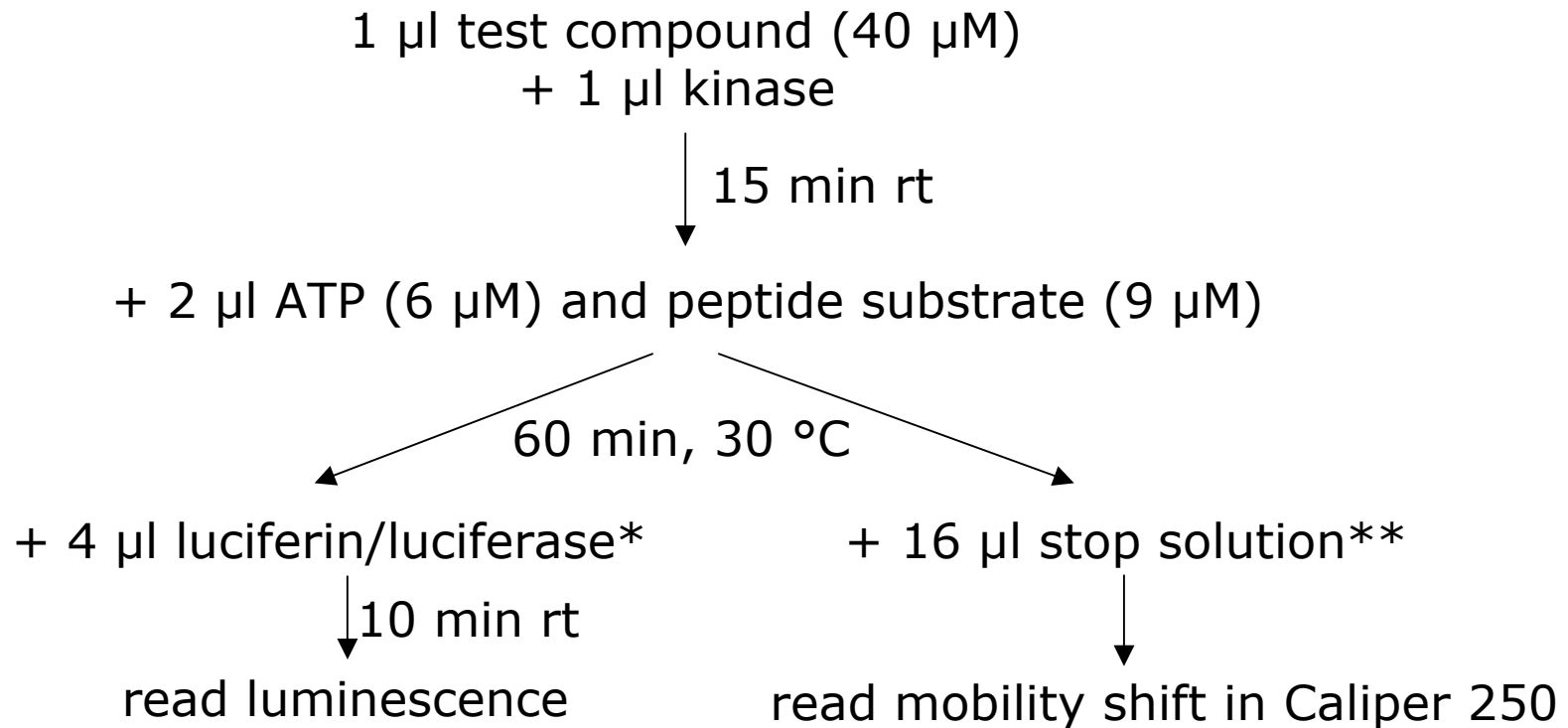
(A)



(B)



## Schematic assay protocols

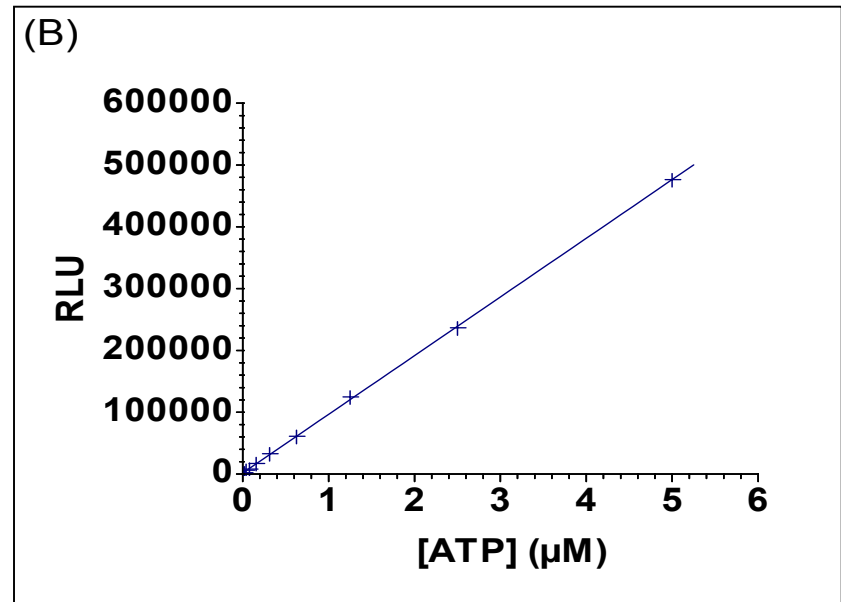
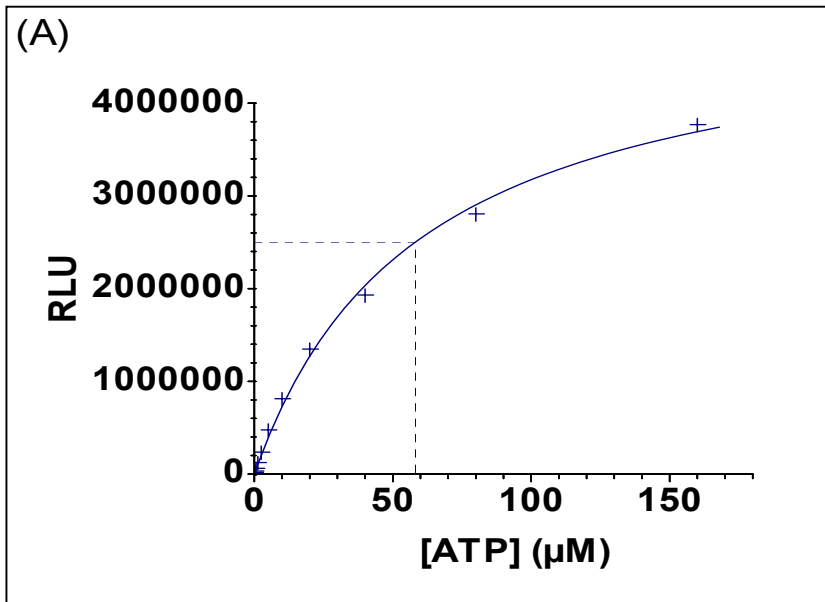


reaction buffer: 25 mM Tris-HCl, pH 7.4, 2 mM MnCl<sub>2</sub>, 10 mM MgCl<sub>2</sub>, 2 mM DTT, 0.02 % BSA  
substrates: RPRAATF, Fluorescein-RPRAATF

\* ATP monitoring solution (Cambrex) plus known inhibitor

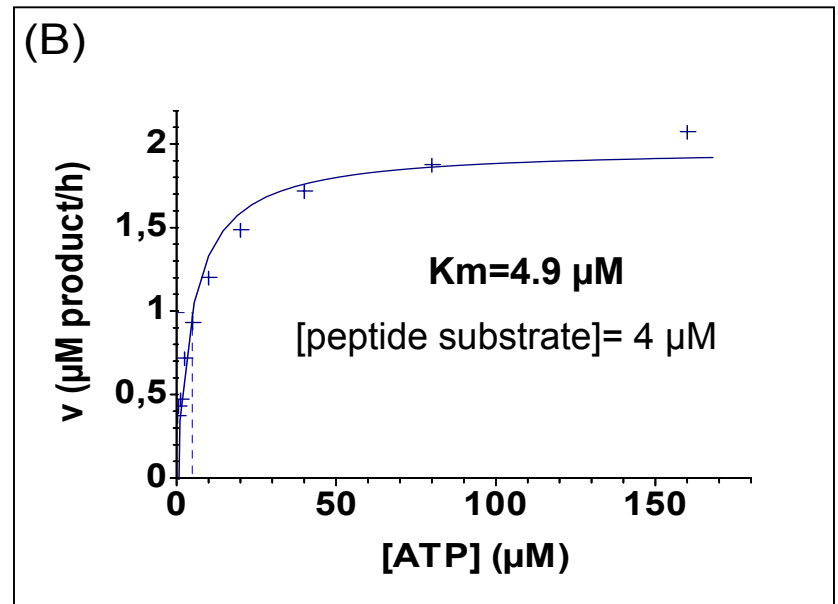
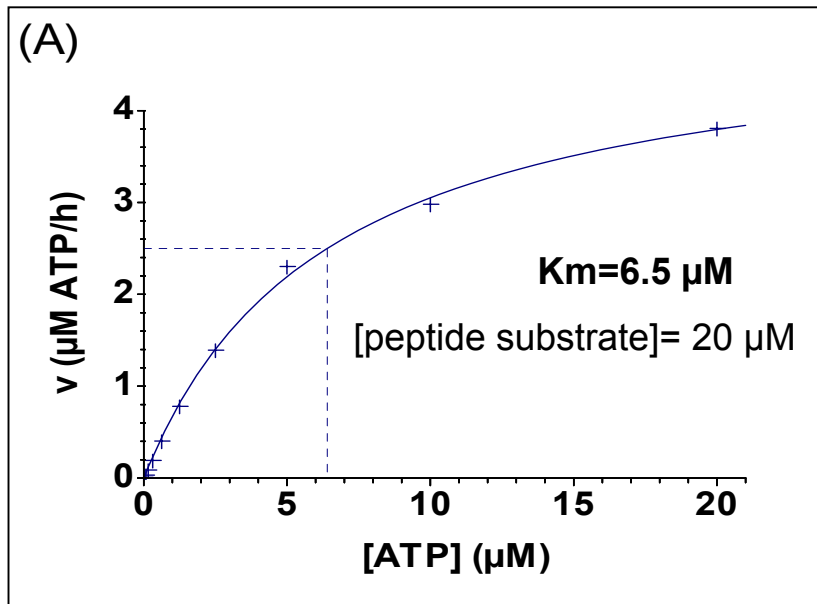
\*\* contains EDTA or known inhibitor

# Bioluminescent assay, signal dependence on [ATP]



⇒ luminescence intensity is directly proportional to [ATP] at concentrations up to 5 μM in the detection volume

# Kinase $K_m$ determination for ATP



$\Rightarrow$  good agreement between the two assay formats

## Screening collection

compound library (1976 compounds)

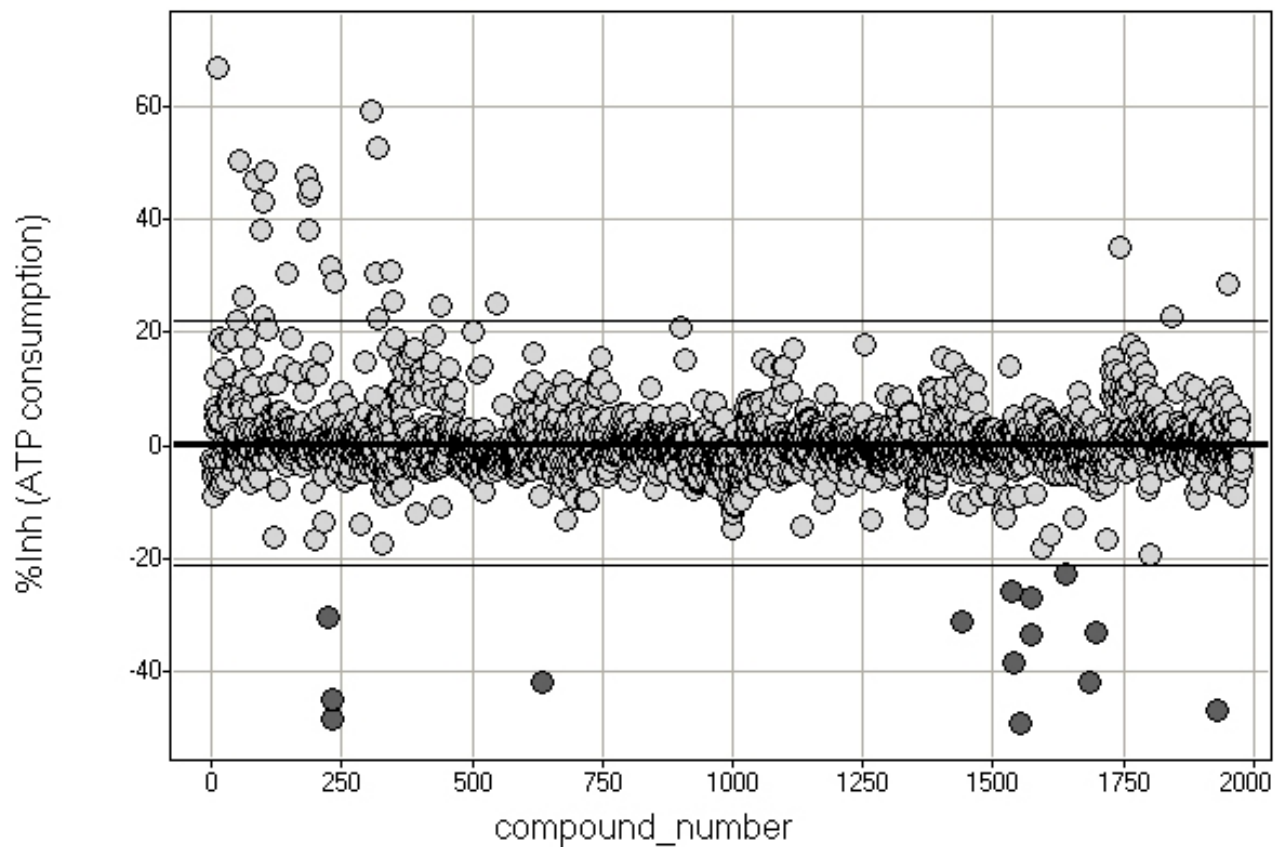
reference set of 157 known kinase inhibitors



2133 compounds

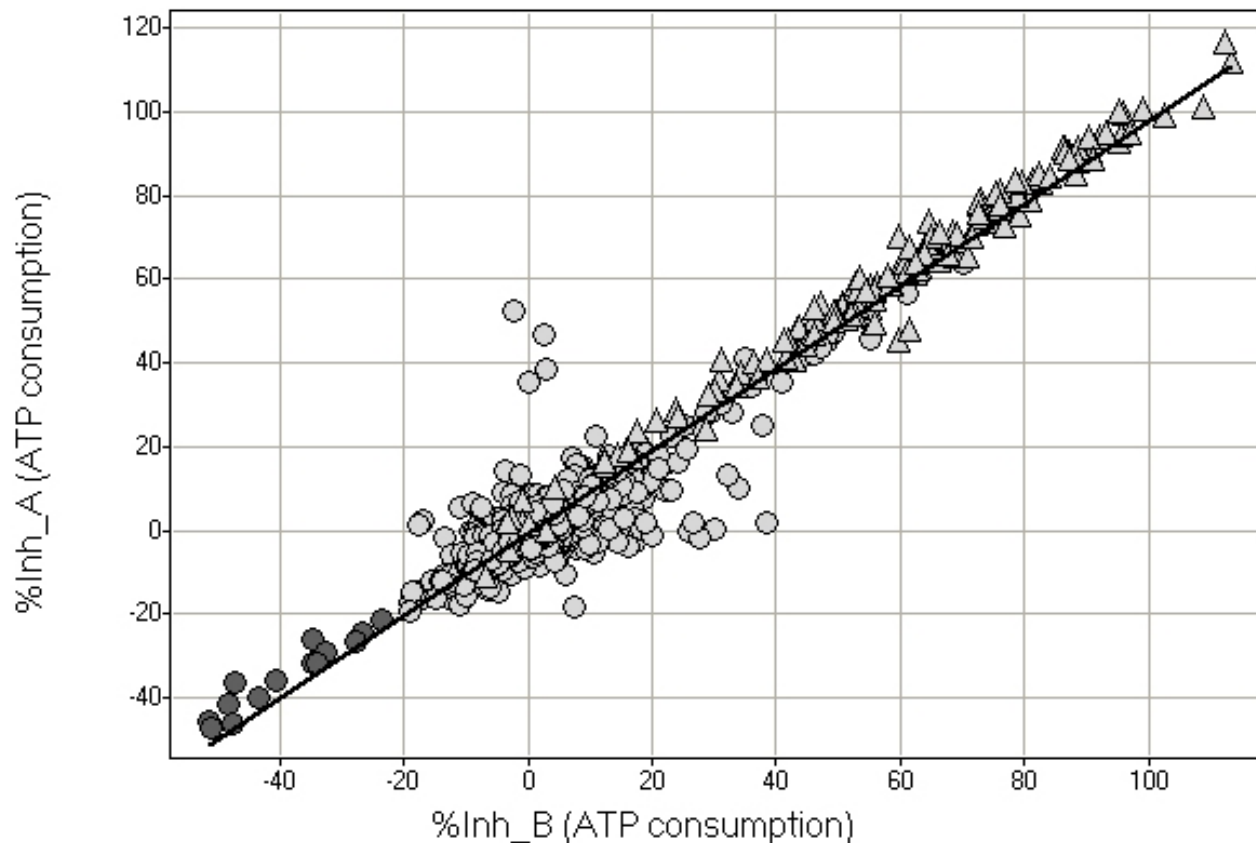
The diagram consists of two arrows pointing towards the number '2133 compounds'. One arrow is a vertical line starting from the text 'compound library (1976 compounds)' and ending in a downward-pointing arrowhead. The other arrow is a diagonal line starting from the text 'reference set of 157 known kinase inhibitors' and ending in a leftward-pointing arrowhead.

# Compound library, ATP consumption assay



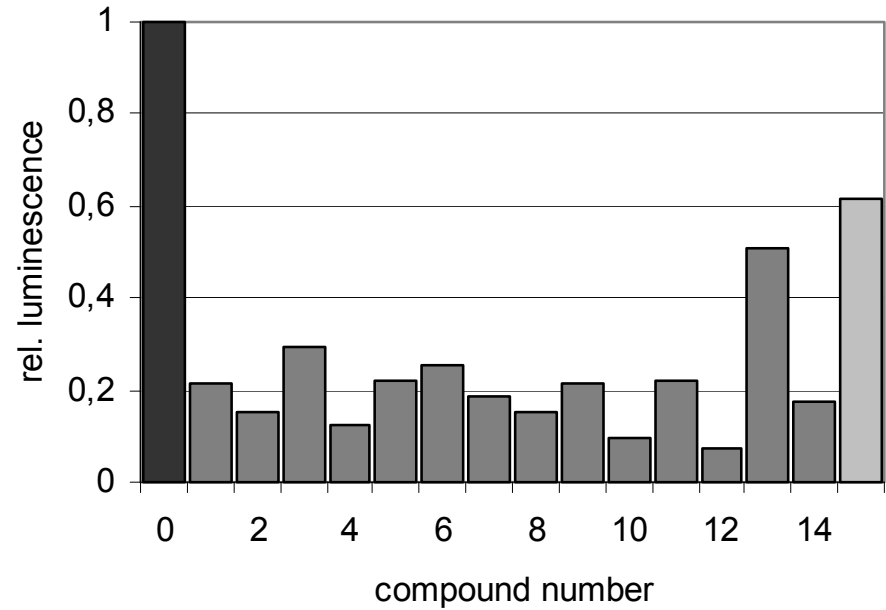
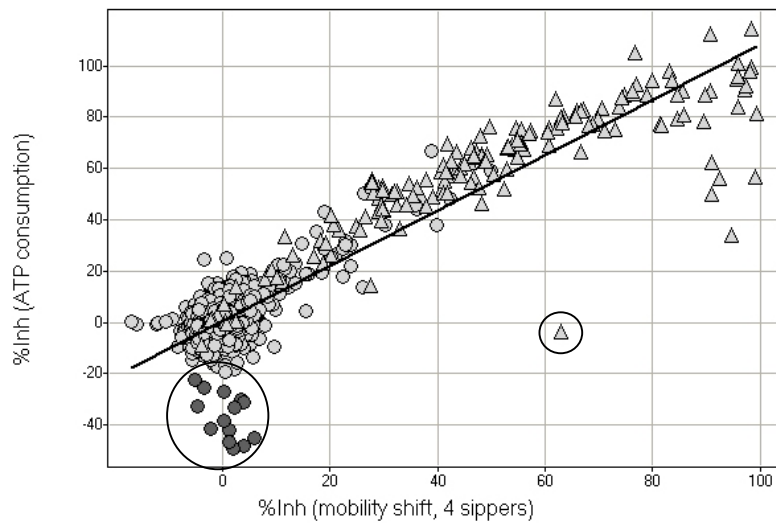
$\mu+3\sigma= 22.2$  percent inhibition

## Correlation of duplicate experiments, ATP consumption assay

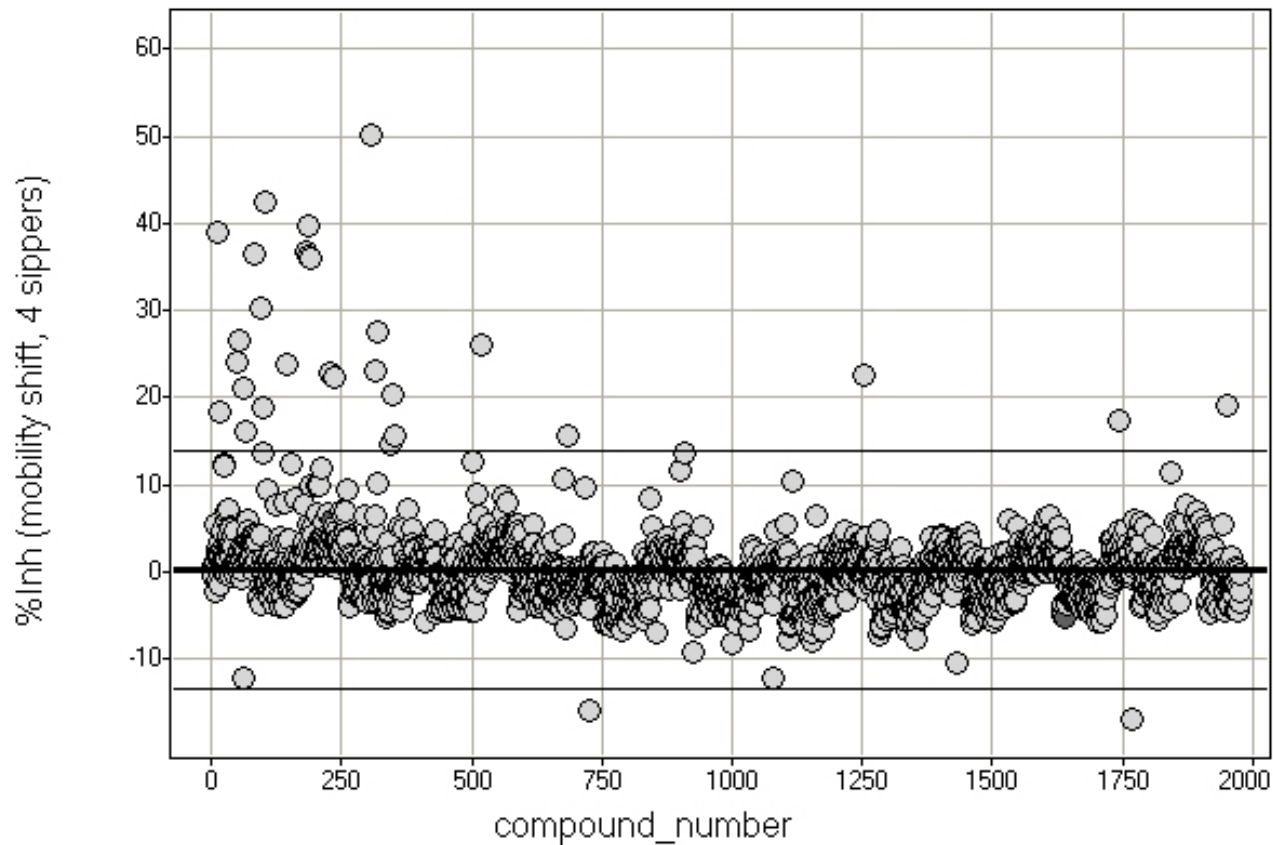


circles: compound library, triangles: reference inhibitor set,  
 $y=mx+n$ ,  $m= 0.95$ ,  $n=1.1$ ,  $R=0.97$

# Luminescence quenchers, incubation without kinase

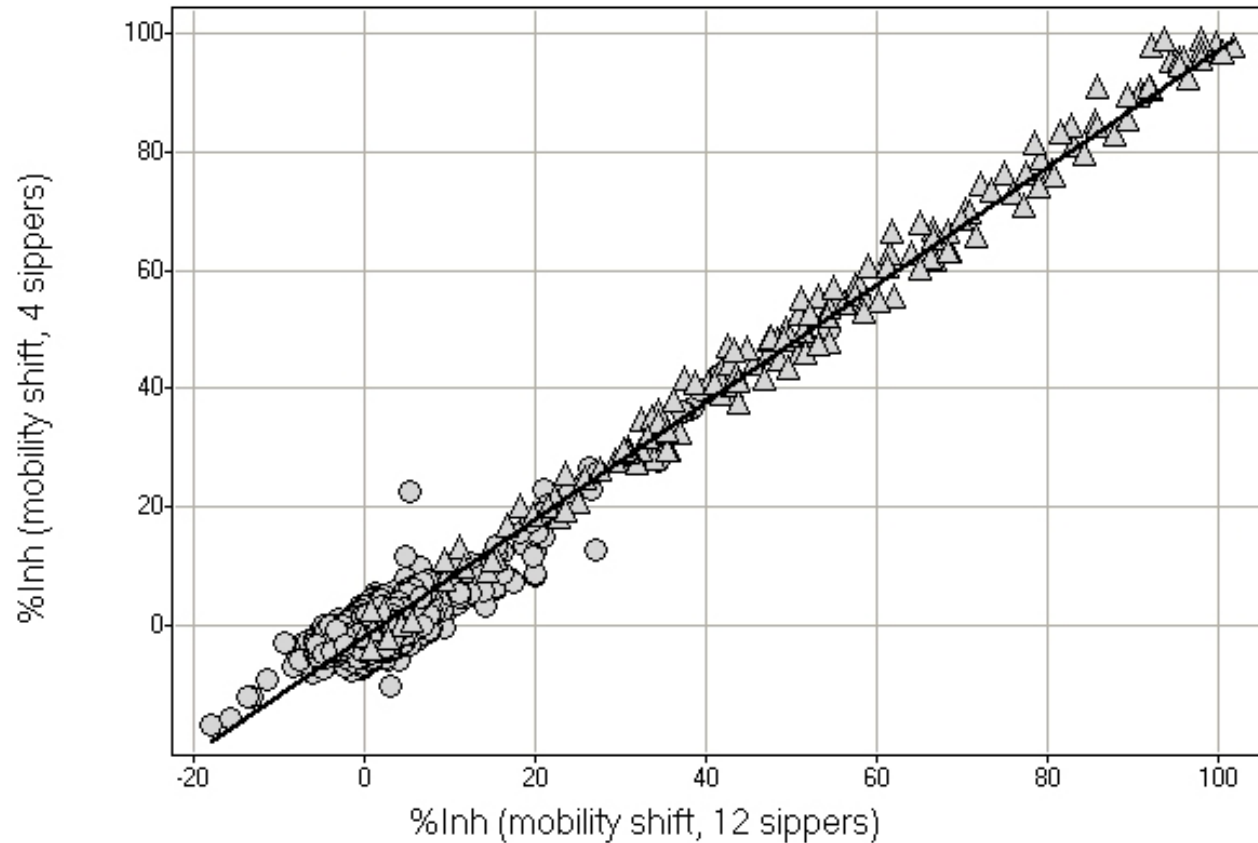


# Compound library, mobility-shift assay



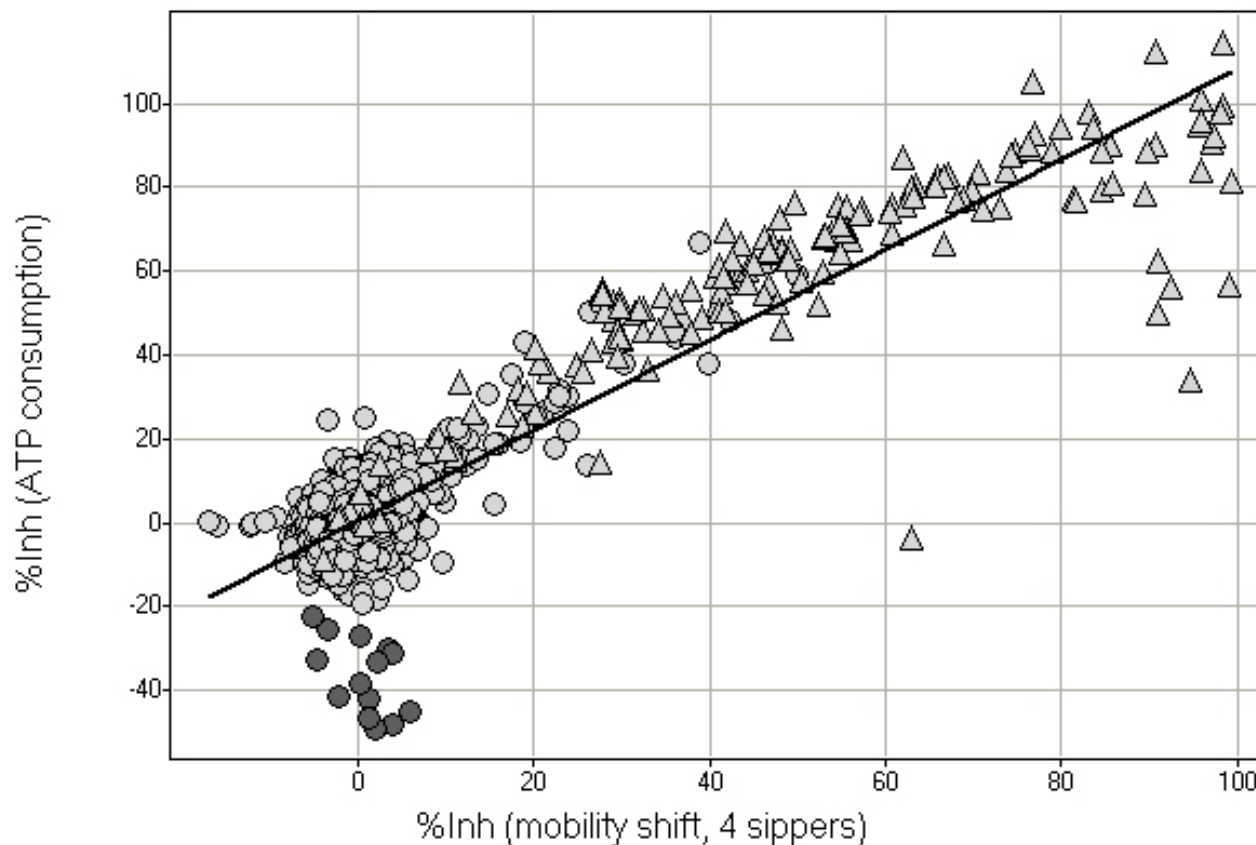
$\mu + 3\sigma = 13.7$  percent inhibition

# Mobility-shift assay, 4-sipper vs. 12-sipper chip



circles: compound library, triangles: reference inhibitor set,  
 $y=mx+n$ ,  $m= 1.0$ ,  $n=-1.9$ ,  $R=0.99$

# ATP consumption vs. mobility-shift assay

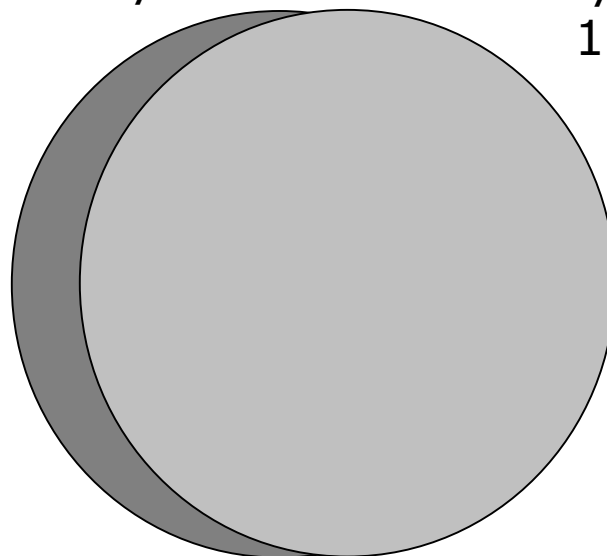


circles: compound library, triangles: reference inhibitor set,  
 $y=mx+n$ ,  $m= 1.1$ ,  $n=0.04$ ,  $R=0.92$

## Overlap of active compounds

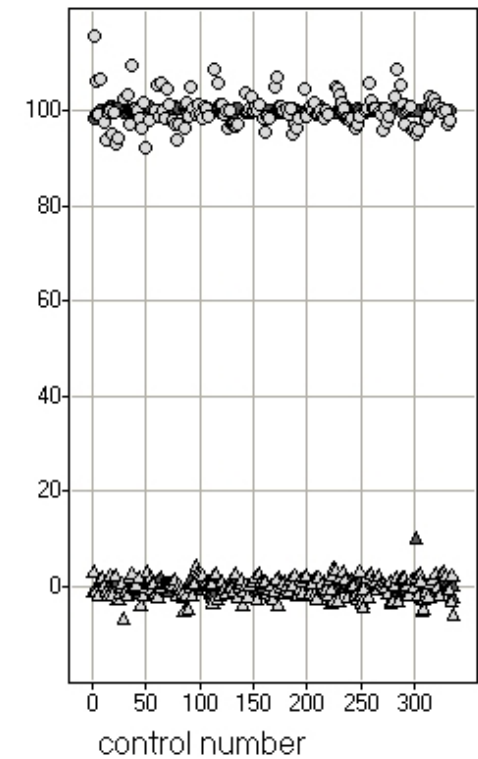
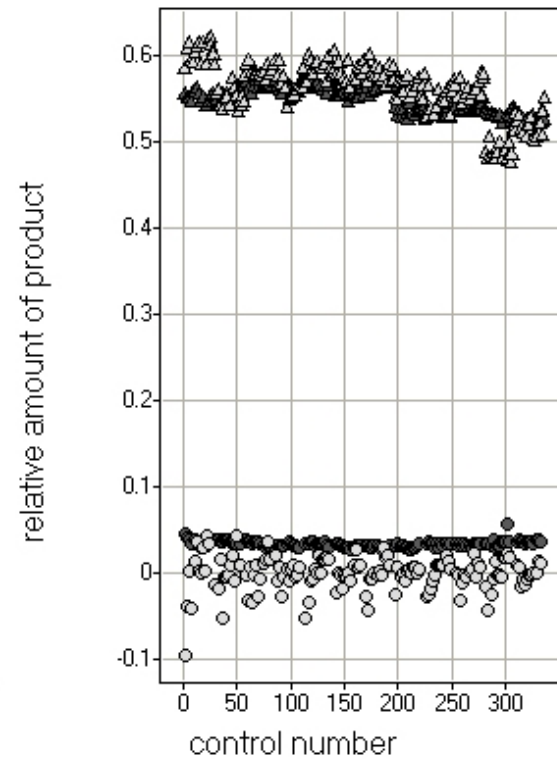
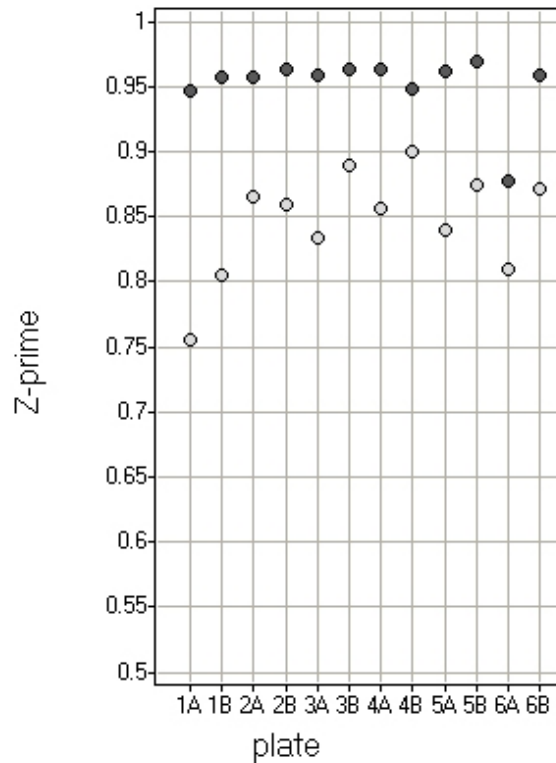
ATP consumption assay  
167 positives

mobility-shift assay  
169 positives



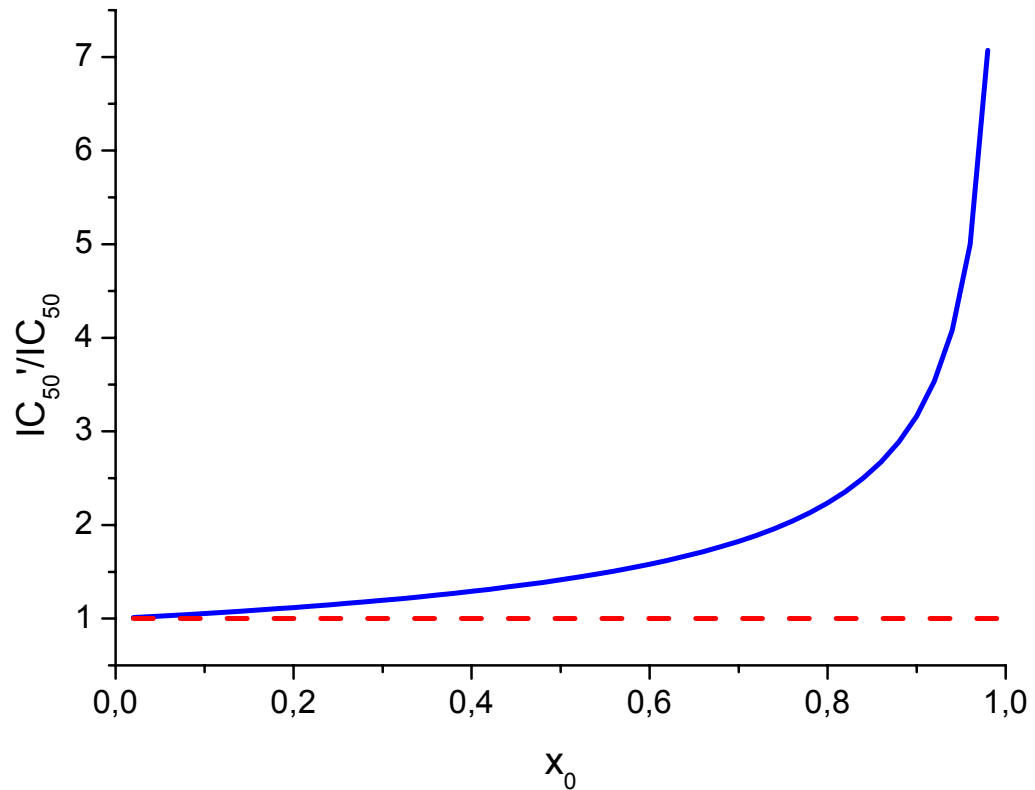
overlap  
160 positives

# Assay quality, controls



dark-grey: mobility-shift assay, light-grey: luminescence assay;  
 SD (low controls) = 1.3 and 2.1 percent inhibition for mobility-shift  
 and bioluminescent assay, respectively

# IC<sub>50</sub> at high substrate turnover



Ref: Wu G, Yuan AY, Hodge CN. Annual SBS meeting, 2002

## ATP consumption assay, pros and cons

### **Advantages**

- substrate variability (proteins, peptides, lipids)
- no fluorescent label required
- no antibody required
- homogeneous, miniaturisable, high throughput
- inexpensive reagents

### **Limitations**

- $[ATP] < 5 \mu M$  in detection volume
- $[\text{phosphorylation substrate}] \geq [ATP]$

⇒ suitable for substrate identification and screening of large compound libraries at low  $[ATP]$

## Mobility-shift assay, pros and cons

### **Advantages**

- antibody-independent
- homogeneous and miniaturisable
- low-noise, high quality data
- little compound interference, low level of false positives or false negatives
- no upper limit on [ATP]

### **Limitations**

- restricted to peptide substrates
- special instrument required, lower throughput

⇒ suitable for screening of focussed libraries and compound profiling or characterisation at different [ATP]

## Acknowledgements

### Cambrex:

- Alex Batchelor
- Lee Walker

### Caliper:

- Holger Schulz
- David Dawson
- Donald McRorie