

# Caliper Profiling Service in GNF Drug Discovery

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GNF



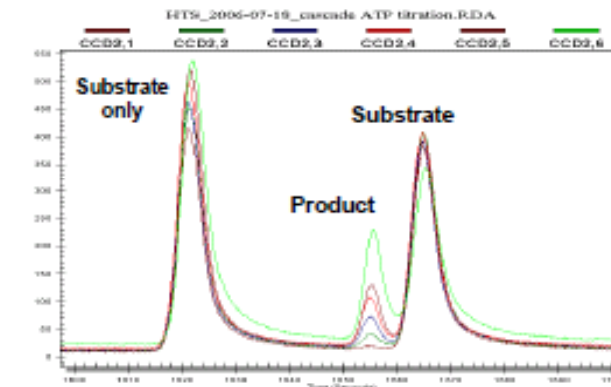
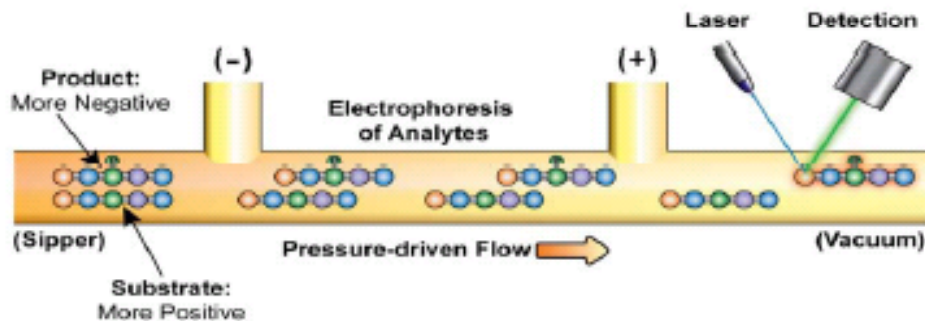
# GNF outsourcing strategies

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- For all targets, we develop in-house biochemical and cellular assays
- For selectivity counterscreens, we decided to outsource this work:
  - The counterscreen panel needs to be adaptable to a changing target portfolio
  - Only a limited number of compounds from several projects are tested in panel
  - Maintaining a large in-house counterscreening panel would require a substantial commitment of resources to setup and run
  - Need the servicer to provide a high degree of flexibility
  - It is economically more beneficial to outsource part of the counterscreening

# Why Caliper?

- Principle: mobility-shift assay



- Activity-based assay that can detect inhibitors with all different modes of inhibition
- Simultaneously measures the levels of both product and substrate resulting in high quality data that is easy to interpret
- Real time kinetics
- Complementary to our in-house HTRF technology
- Drawbacks: a relatively small panel of kinases (~140).

# Collaboration with Caliper

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- Caliper reps: Natalie McCan and Mike Marlowe
- Validation study:
  - Side-by-side studies with Invitrogen and Caliper (~50 compounds)
  - Compare Invitrogen/Millipore/GNF data to the Caliper data
  - The quality of the Caliper are satisfactory
- Contract term
  - Reasonable cost per well
  - Flexible enzyme panel selection
  - No commitment on the volume of usage (“pay as you go”)
  - Two week data turn around time

# GNF sub-panel for Caliper profiling

- We selected a panel of 51 kinases to be run by Caliper
  - We do not want to test every available kinase
  - A total of ~100 kinases (between Caliper and in-house combined) should be representative of a compound's selectivity
  - Selected kinases include targets, target family members, class representatives, “must-hit's”, and “must-not-hit's”
  - Profiling is done as single-point % inhibition at a fixed compound concentration in duplicate. IC50 follow-up is done if needed.
- Profiling is for early stage compounds to understand selectivity issues
- For late-stage drug candidates, more extensive profiling services such as Ambit will be used

Assay Name	Assay Name
Abl (h)	JNK2 (h)
AurA (Aur2/STK6) (h)	KDR (VEGFR2, FLK1) (h)
AurB (h)	Lck (h)
Axl (h)	LynA (h)
BMX (h)	MAPK1 (ERK2, p42mapk) (h)
BTK (h)	MAPK13 (p38delta, SAPK4) (h)
c-Kit (h)	MAPK14 (p38alpha, SAPK2alpha) (h)
CDK2/cyclinA (h)	MEK1
cRAF (RAF1) (h)	MET (h)
CSK (h)	NEK2 (h)
DYRK1a (h)	p70S6K (h)
EGFR (ErbB1) (h)	PDGFRa (h)
EphA3 (h)	PDGFRb (h)
EphB3 (h)	Pim2 (h)
ErbB4 (HER4) (h)	PKC-alpha (h)
FAK2 (PYK2/PTK2b) (h)	PLK1 (h)
FGFR3 (h)	RET (h)
Flt3 (h)	ROCK1 (h)
FMS (CSF1R) (h)	SGK1 (h)
Fyn (h)	Src (h)
GSK-3beta (h)	Syk (h)
IGF1R (h)	TrkA (NTRK1) (h)
IKKb (h)	TTK (h)
InsR (h)	TYRO3 (h)
IRAK4 (h)	ZAP70 (h)
Jak2 (h)	

# Flexibility of the Caliper profiling service

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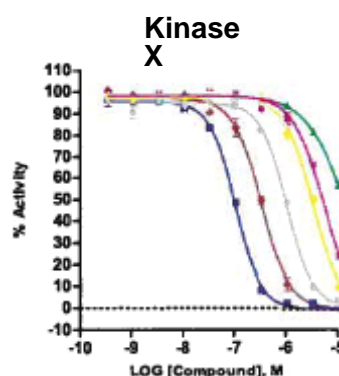
- The Caliper profiling service is flexible, and accommodates our various requests:
  - Single point % inhibition test
  - IC50 request for any enzyme on the Caliper panel
  - MoA studies of the compounds, for example:
    - Time-dependent inhibition
    - Reversibility/Dissociation kinetics
    - ATP competition

# Good data quality

- So far, the data from the Caliper profiling service has been high quality, reproducible, and shows concordance with in-house data
  - Caliper data can generally be confirmed by analogous in-house assays

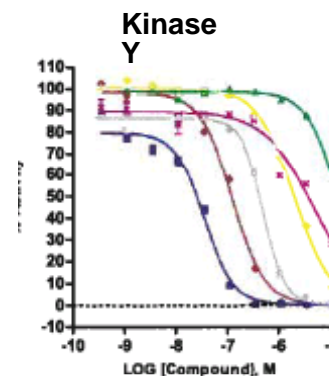
## Time-Dependent Inhibition Studies

		Kinase X IC50 (uM)	
Compound	Pre-incubation time (min)	0	60
GNF-YJ1	Caliper	0.3	0.09
	GNF	0.5	0.01
GNF-YJ2	Caliper	No Fit	4.9
	GNF	46	1
GNF-YJ3	Caliper	3.1	1
	GNF	5.6	0.1



	HillSlope	IC50
GNF-YJ1 (No Preinc)	-1.6	3.1e-007
GNF-YJ1 (60 min Preinc)	-1.8	9.8e-008
GNF-YJ2 (No Preinc)		
GNF-YJ2 (60 min Preinc)	-1.5	4.9e-006
GNF-YJ3 (No Preinc)	-1.6	3.1e-006
GNF-YJ3 (60 min Preinc)	-1.8	1.0e-006

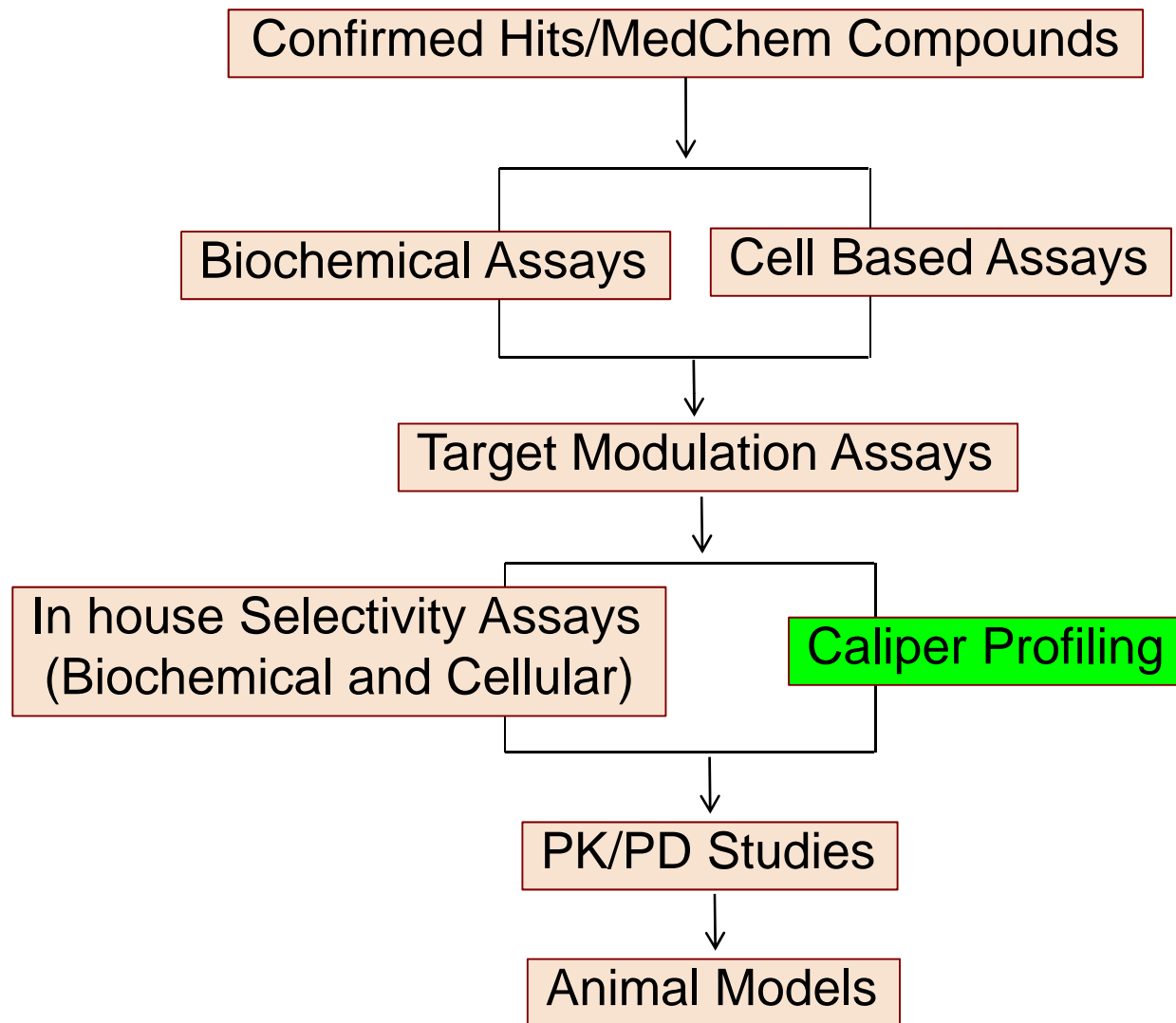
\* % Activity does not reach 50%:  
IC<sub>50</sub> not calculated



	HillSlope	IC50
GNF-YJ1 (No preinc)	-1.4	1.1e-007
GNF-YJ1 (60 min preinc)	-1.5	3.2e-008
GNF-YJ2 (No preinc)		
GNF-YJ2 (60 min preinc)	-0.89	4.5e-006
GNF-YJ3 (No preinc)	-1.2	1.8e-006
GNF-YJ3 (60 min preinc)	-2.0	4.8e-007

\* % Activity does not reach 50%:  
IC<sub>50</sub> not calculated

# How Caliper data fits into GNF project flow chart



# Caliper data follow-up

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- The main end users of the selectivity data are both chemists and biologists
- When selectivity issues arise, we:
  - Request a compound dose-response (IC50) from Caliper
  - Conduct analogous dose-response assays in-house (if available)
  - Expand the SAR to see if the selectivity can be addressed synthetically
  - Develop biochemical and cellular assays in-house to allow for continuous monitoring of the selectivity problems
  - Try to be opportunistic: target, panel, compound series

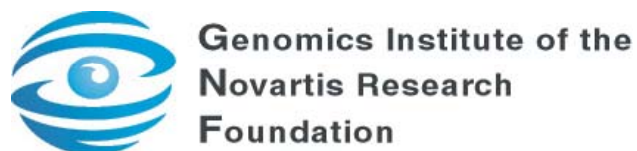
# Looking forward...

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- GNF has established a mutually favorable collaboration with the Caliper profiling service
- Benefits of the Caliper Profiling service:
  - Technology
  - Good data quality
  - Flexibility, accommodating, willing to work together
- Areas that need improvement:
  - Data turnaround time (sometimes >2 weeks)
  - Partial data package
- We look forward to continuing this productive relationship!

# Acknowledgement

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All chemists and biologists

Jennifer Harris

Martin Seidel

Pete Schultz

Natalie McCan

Mike Marlowe

Peter Franks

Benjamin Lineberry

Other bench scientists

