

USING OLD INHIBITORS TO START CHEMICAL PROBE DEVELOPMENT ON NEW TARGETS: NAK FAMILY KINASES

New Horizons in Medicinal Chemistry of Protein Kinases Workshop

Jon Elkins

FAPESP, 7th March 2016



THE UNIVERSITY
of NORTH CAROLINA
at CHAPEL HILL

SGC UNC



SGC Toronto

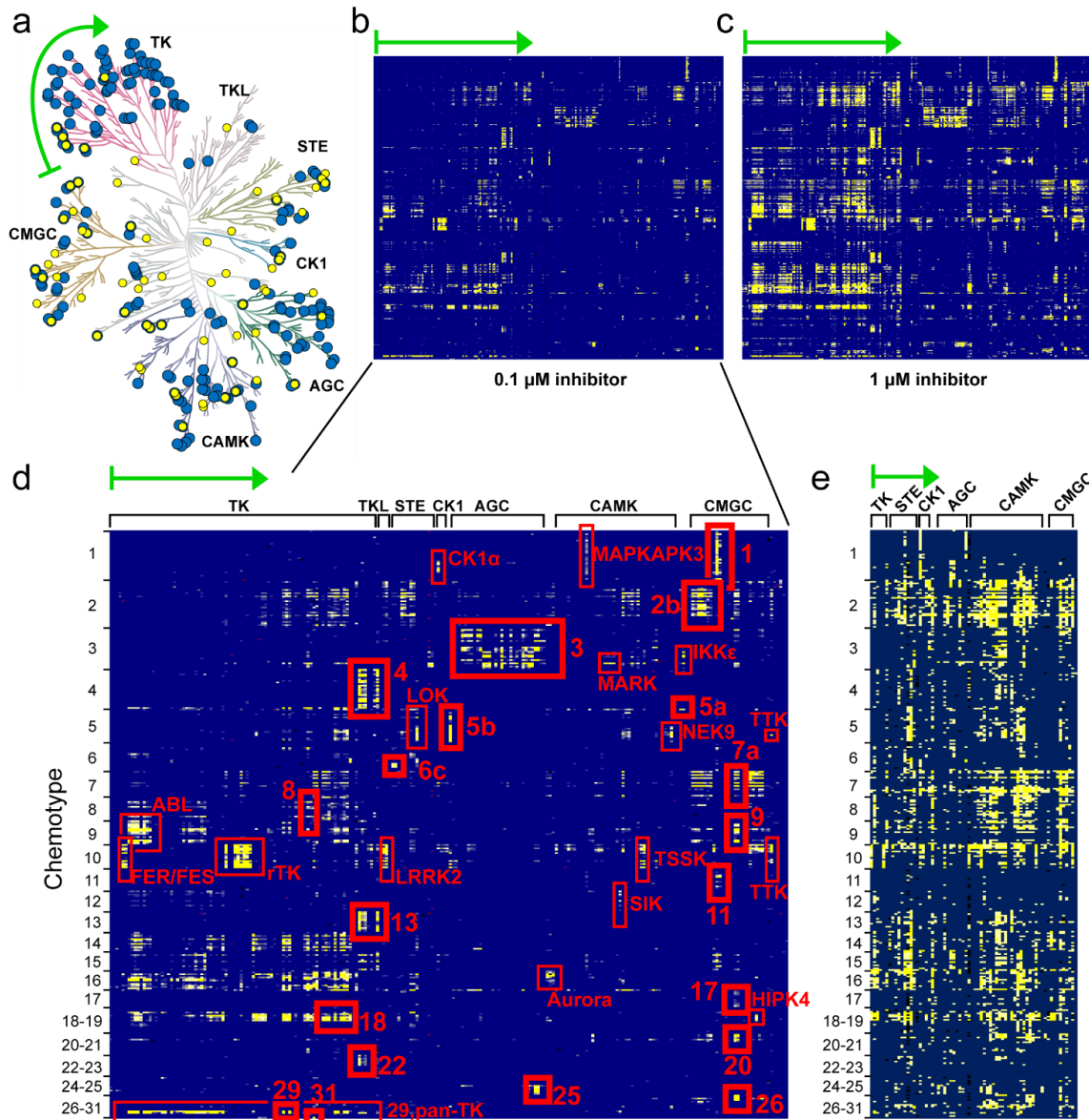


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367 compounds

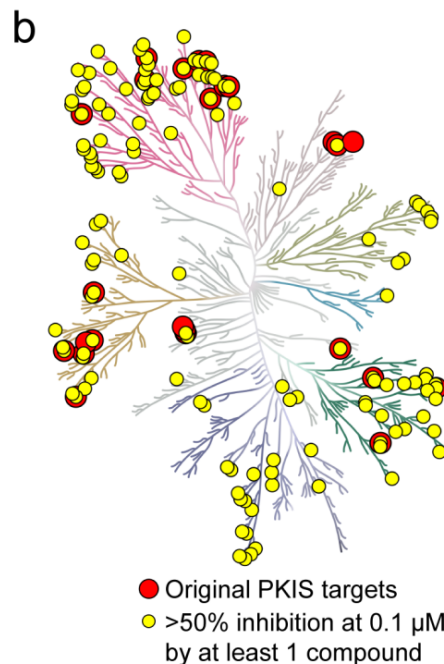
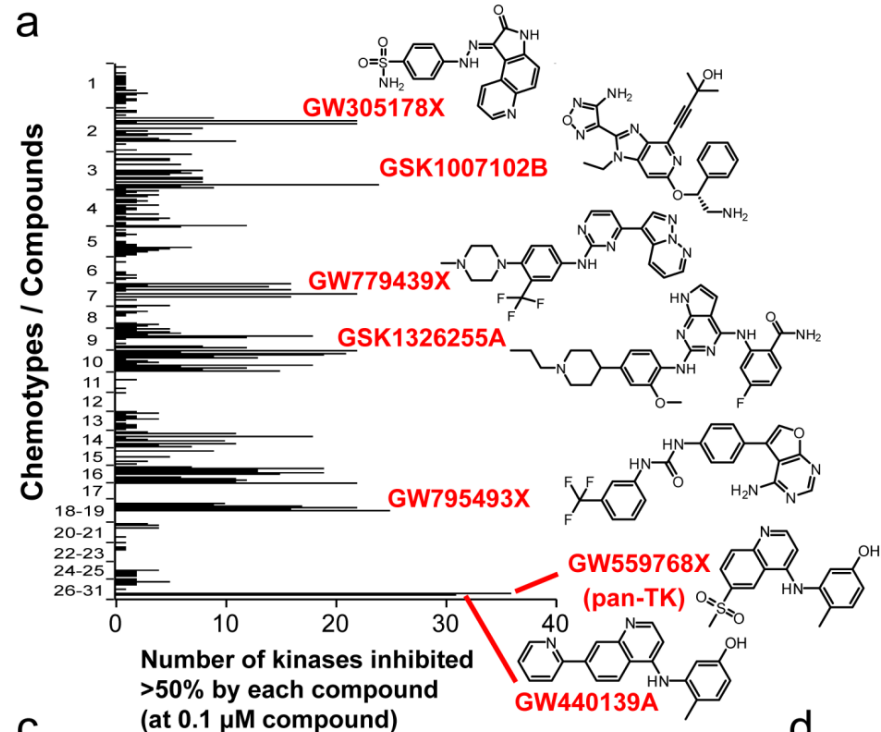
~31 chemotypes

Screened against:

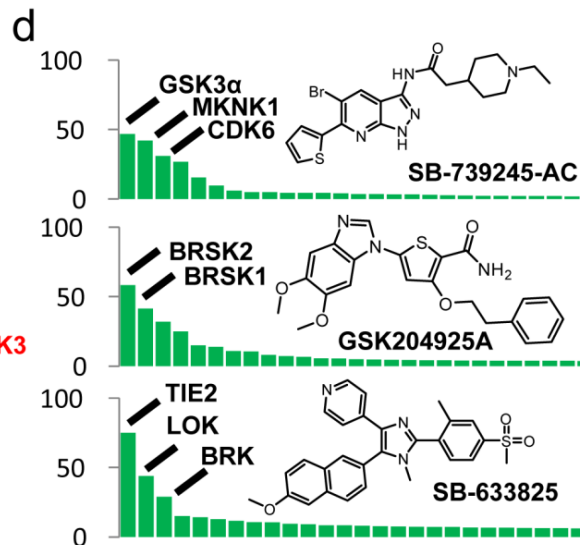
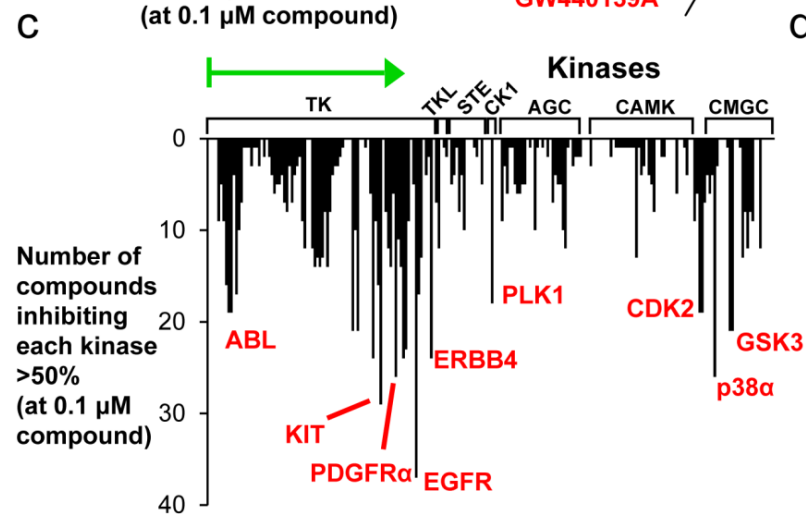
224 kinases (enzymatic assay)

70 kinases (DSF)

= original target of chemotype
 = alternate targets observed for chemotype
 a,b,c = different original targets of the same chemical series, where these targets are not closely related.
 0%  100% inhibition

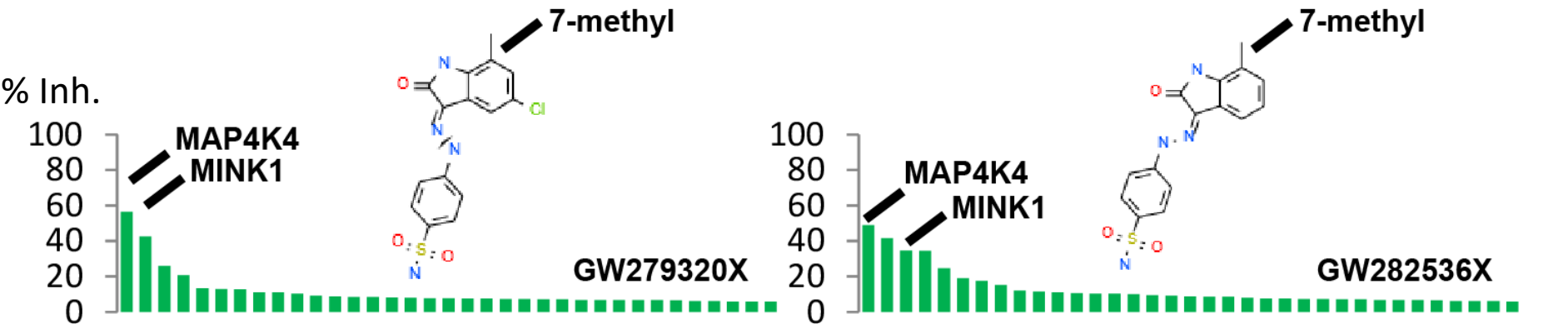
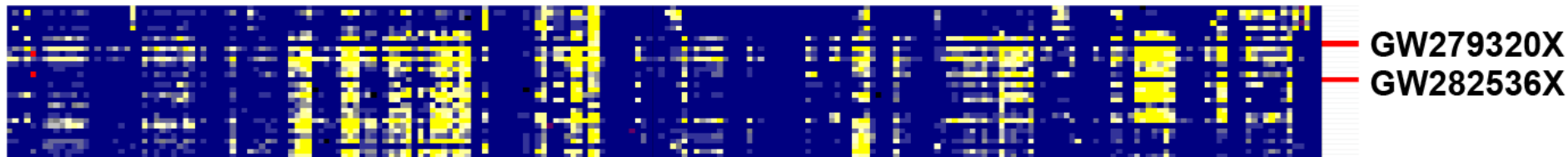


Large variety of novel targets identified

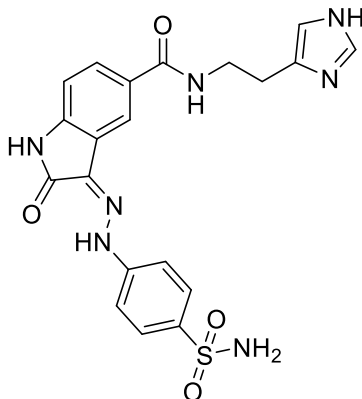
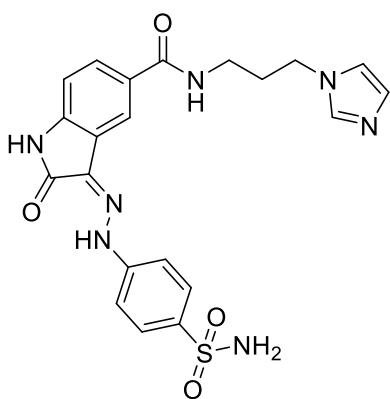


And some chemical probe starting points

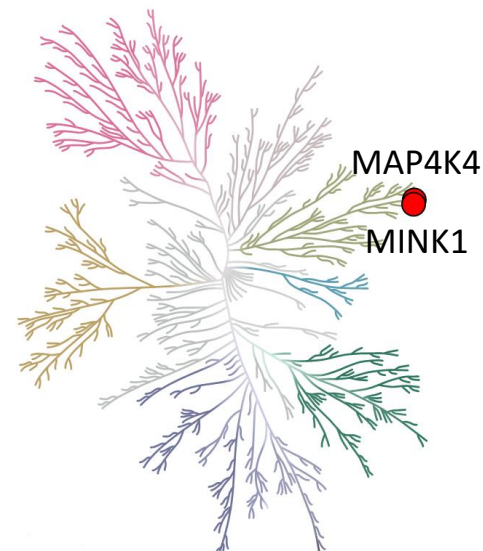
Oxindole chemotype examples

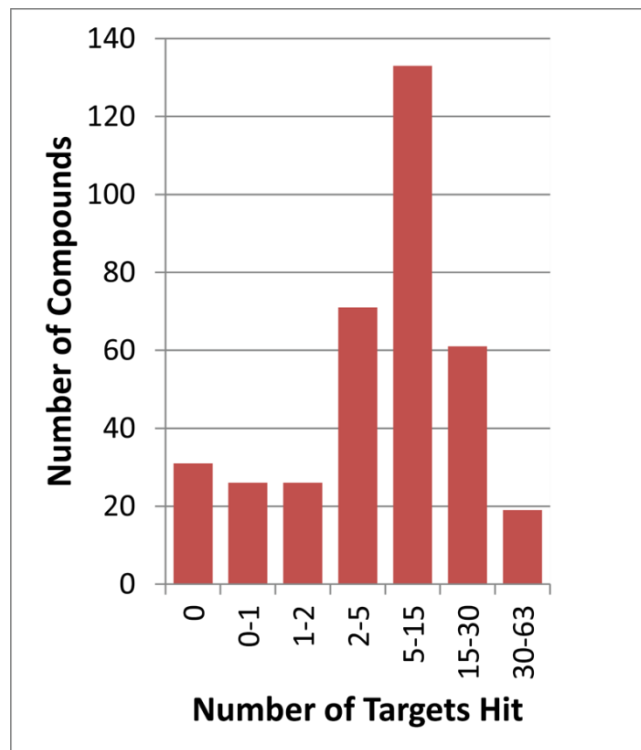
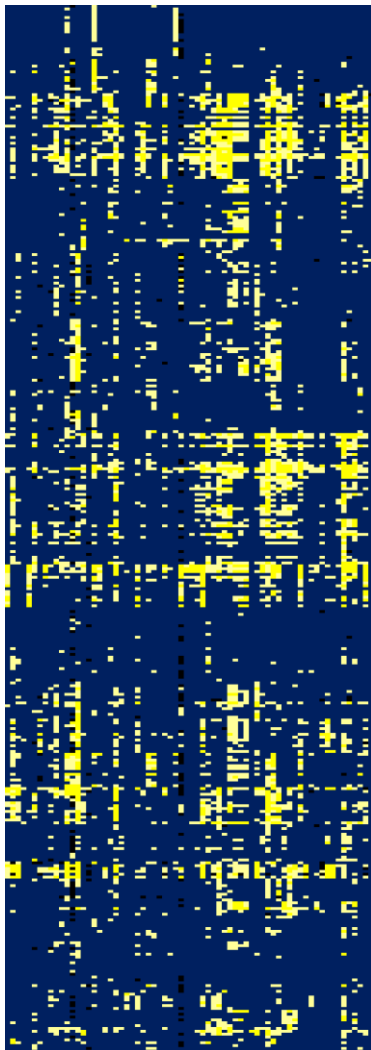


7-methyl makes oxindoles specific for MAP4K4/MINK



FER Activators



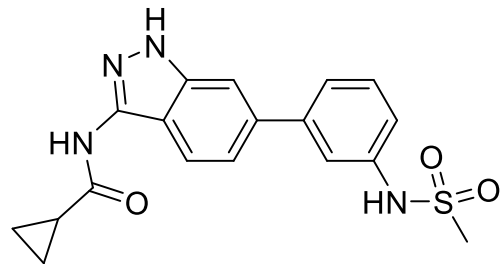


Correlations with the enzyme assay data (26 Targets in common):

0.1 μM - correlation 0.45

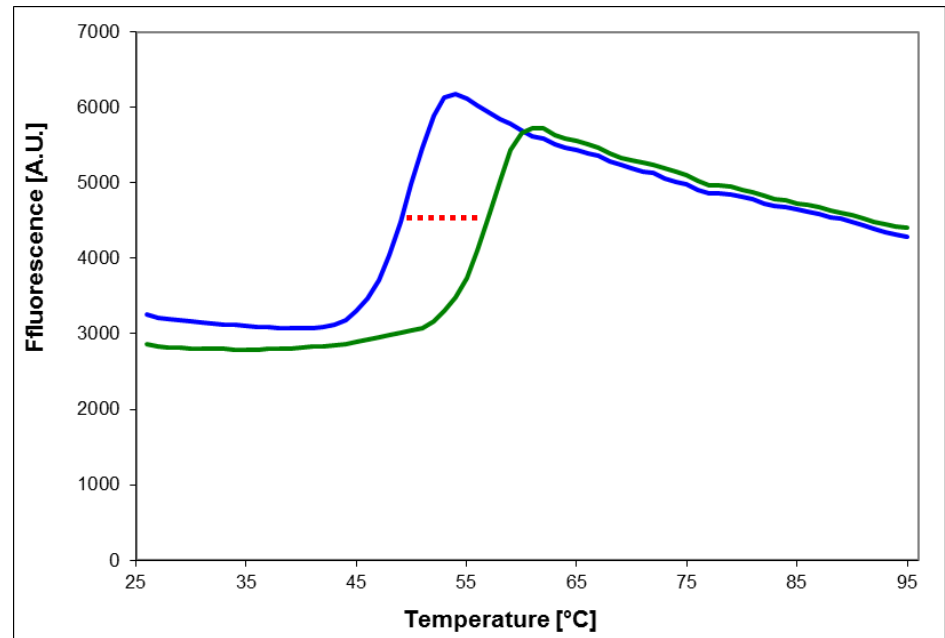
1.0 μM - correlation 0.64

SGC screening was done at 10 μM compound



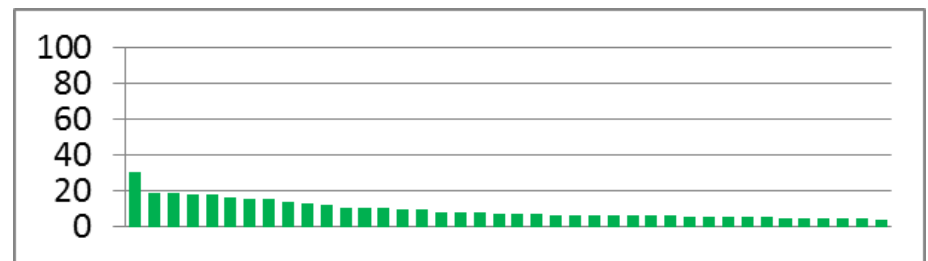
SB-742864

$\Delta T_m = 7.1\text{ }^\circ\text{C}$



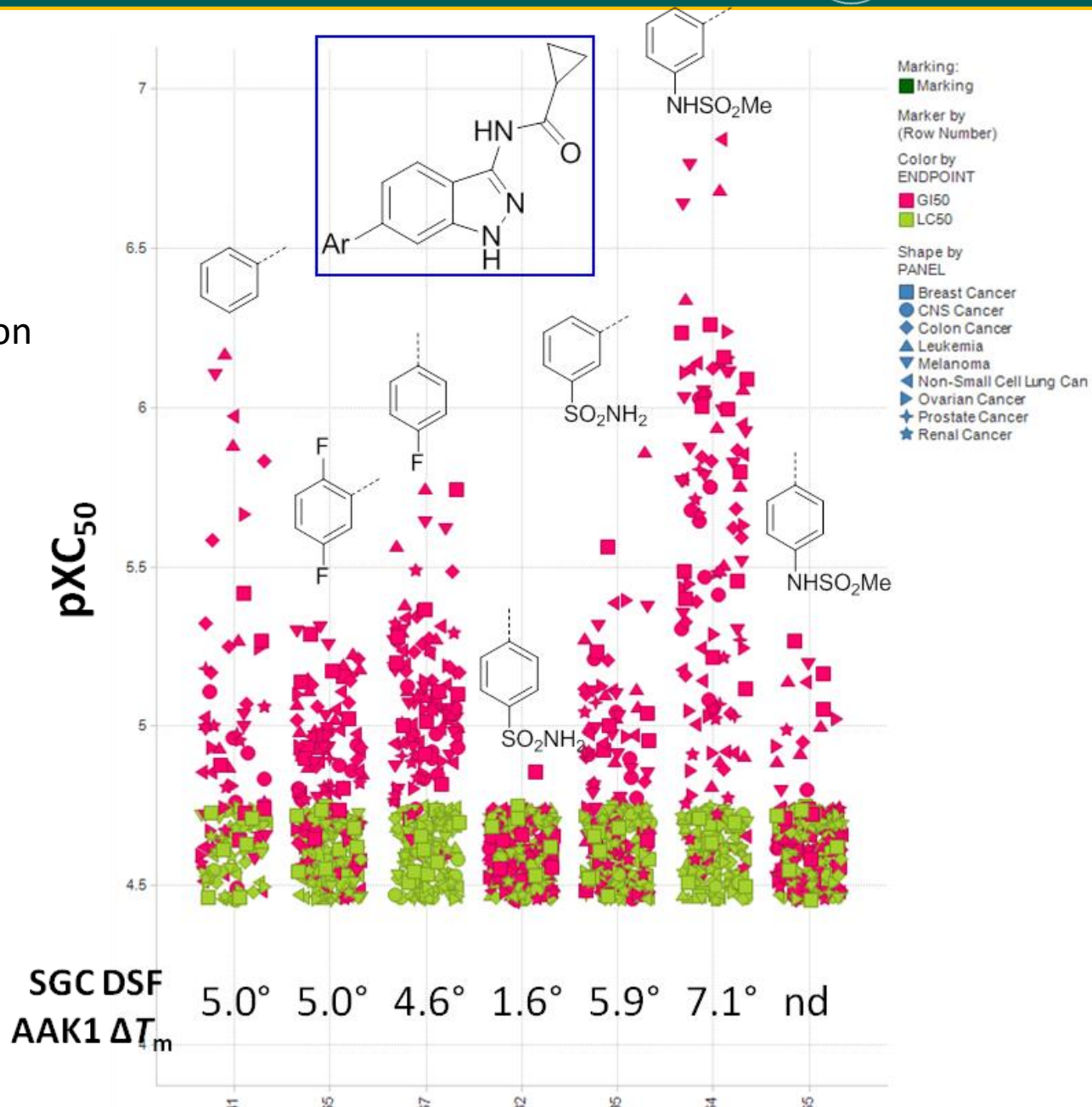
Clean profile in Nanosyn screening data
at 1.0 μM (224 kinases)

Part of a series originally targeting GSK3 β
 $\text{IC}_{50} > 1\text{ } \mu\text{M}$ vs GSK3 β

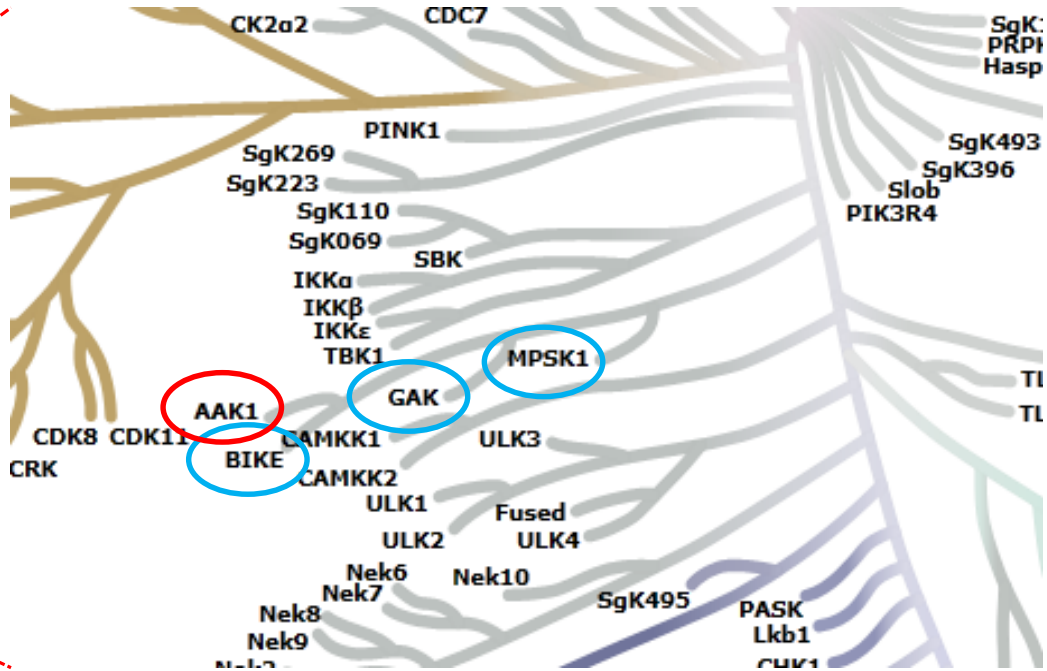
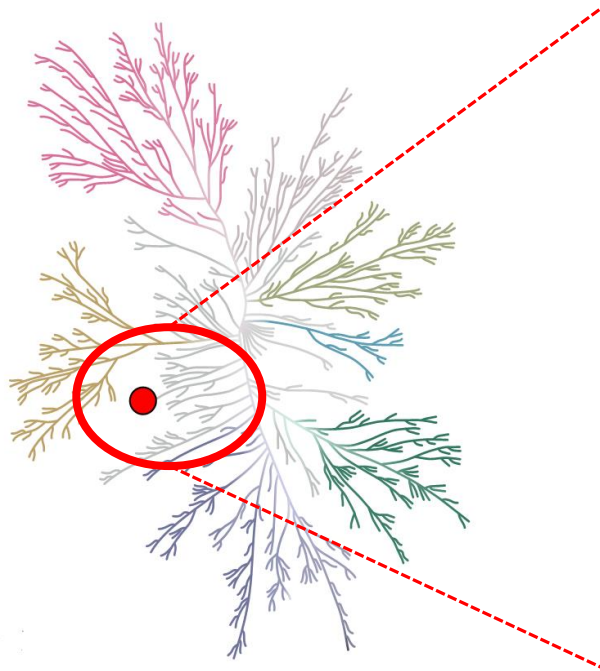


DSF and cancer cell inhibition correlation

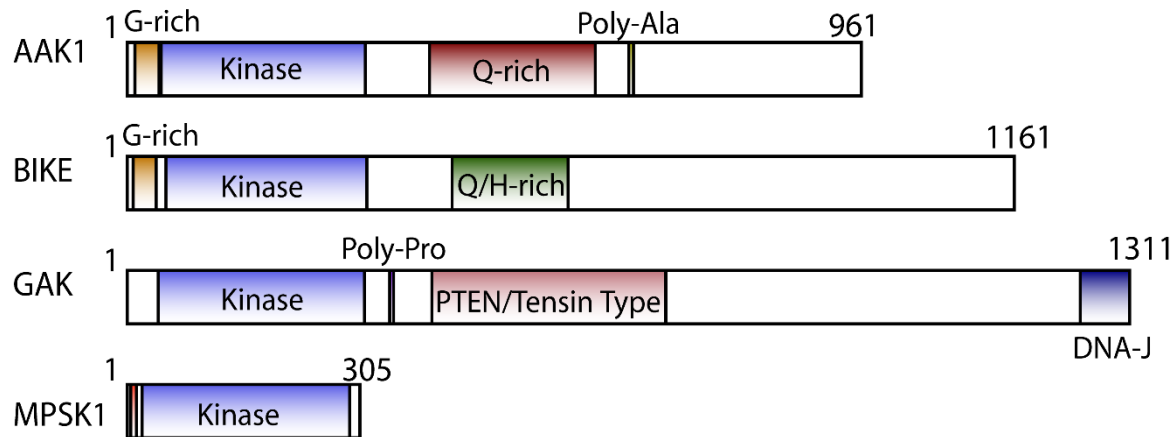
- NCI-60 panel screening
- Low overt toxicity (LC₅₀ all > 20 μM)
- Chemotype shows correlation between ΔT_m and pGI₅₀

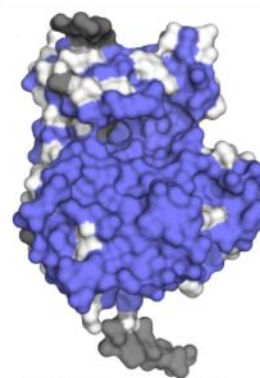
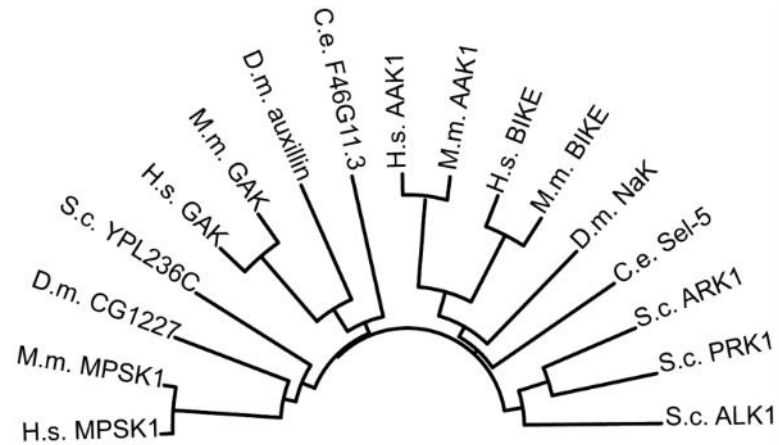
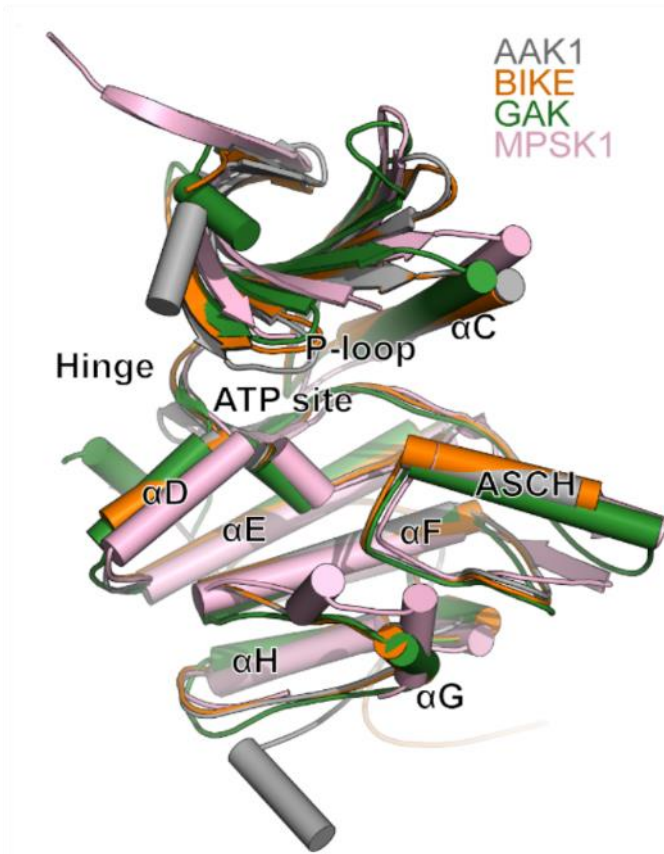


Adaptor protein 2-Associated Kinase 1 (AAK1)

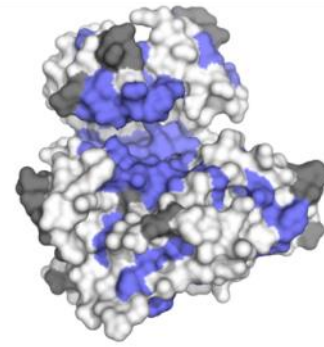


- Only ~30 citations (Pubmed)
- Phosphorylates Adaptor Protein 2 (AP2) and NUMB
- Regulator of endocytosis
- Activity stimulated by clathrin
⇒ Regulator of clathrin-mediated endocytosis
- Part of Numb-Assoiated Kinase (NAK) family

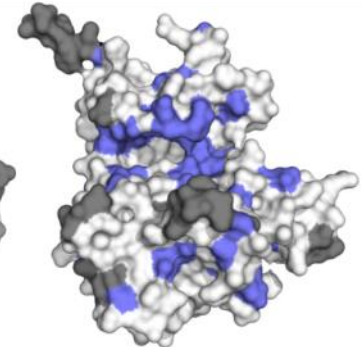




BIKE vs AAK1



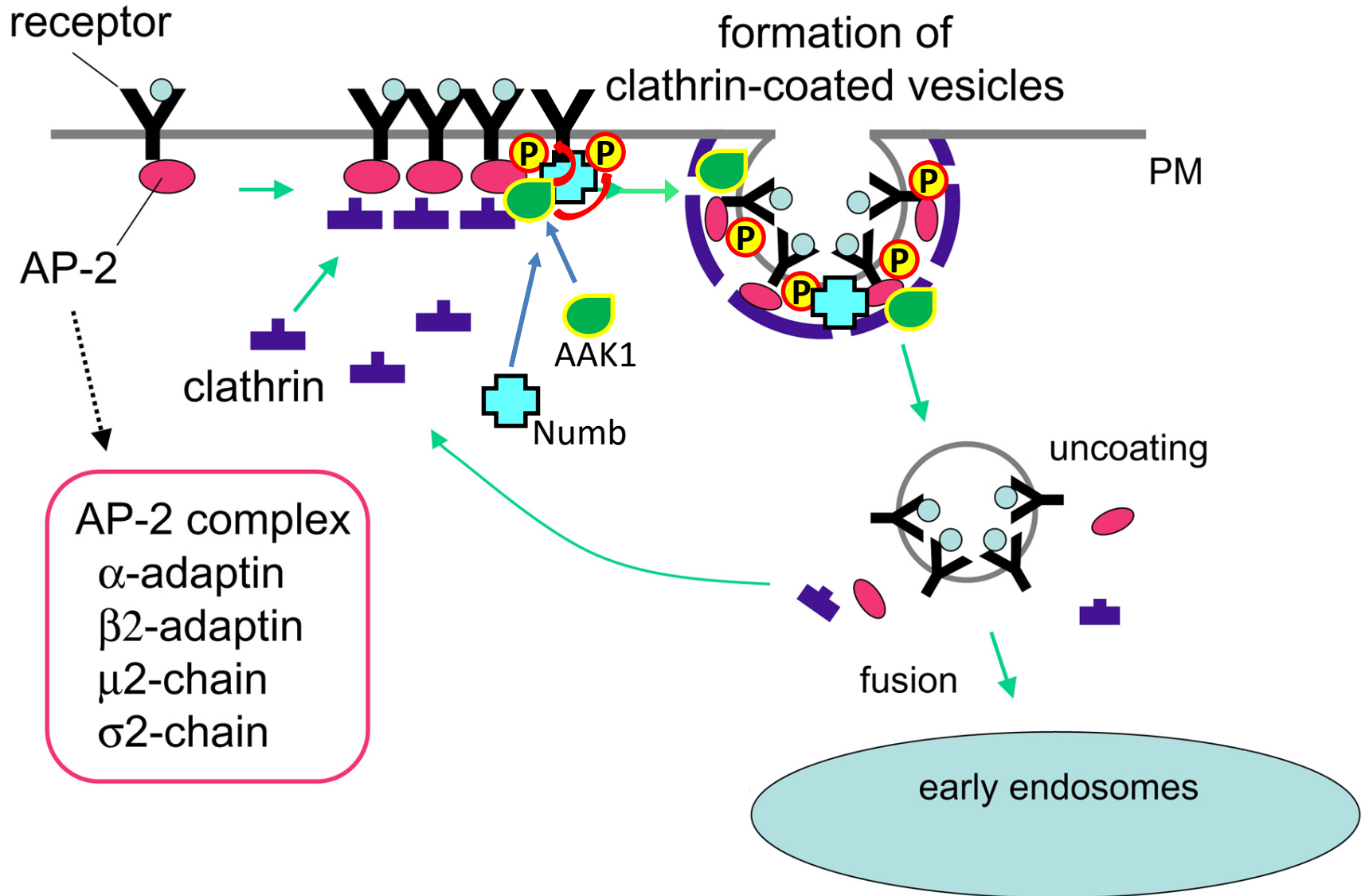
GAK vs AAK1



MPSK1 vs AAK1

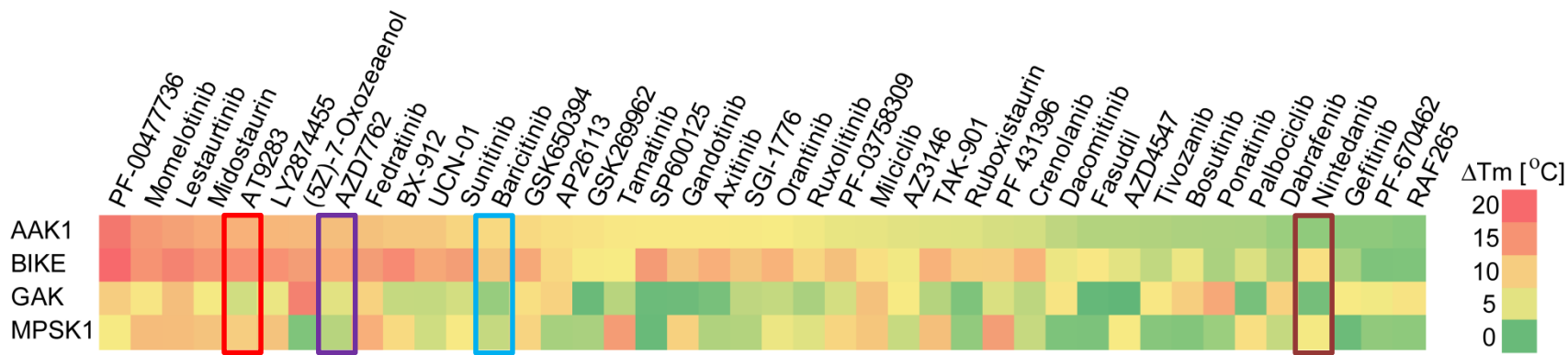
Fiona Sorrell

- AAK1 and BMP2K (BIKE) most closely related to *Drosophila* protein kinase NAK
- Conserved activation segment C-helix (ASCH)
- BMP2K also a clathrin-coated vesicle-associated protein and Numb-associated
- GAK essential for clathrin trafficking and binding to the plasma membrane

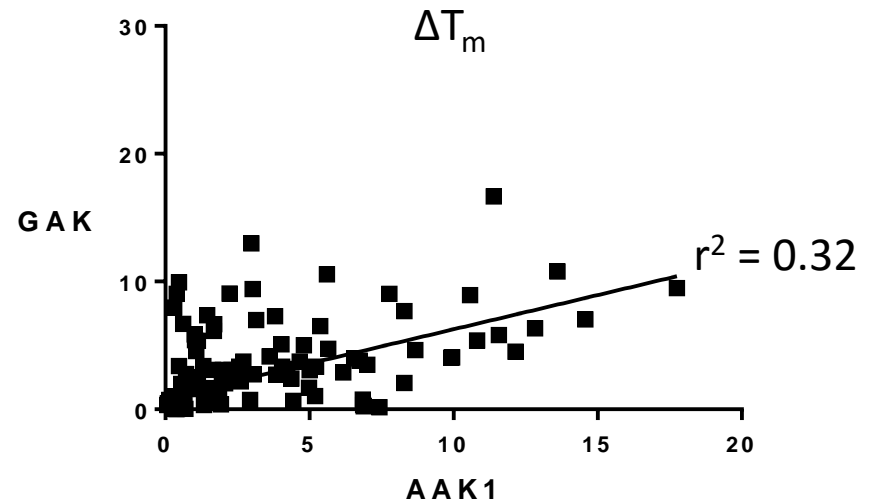
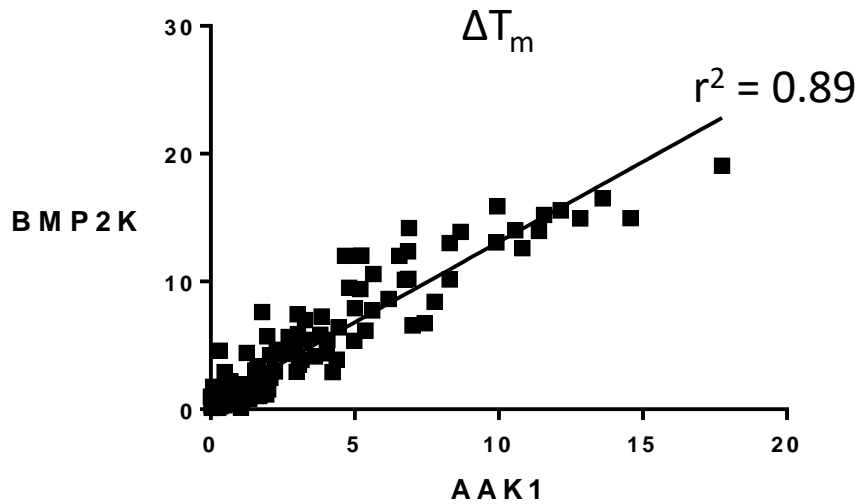


- Superoxide dismutase 1 (SOD1) mutations cause a toxicity that leads to familial form of amyotrophic lateral sclerosis (ALS).
 - AAK1 selectively interacts with mutant SOD1, but not with wild-type.
 - AAK1 Expression is granular in rodent spinal cord.
 - AAK1 mislocated into aggregates with mutant SOD1s and neurofilament proteins in rodent models of ALS.
 - AAK1 levels were decreased in spinal cords of ALS patients.
- => Impaired neurotransmission / vesicle recycling.

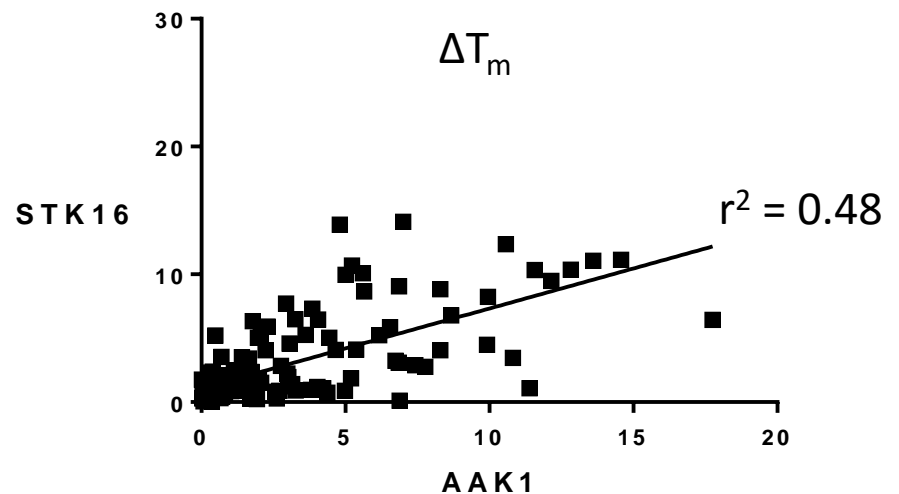
NAK family with clinically used inhibitors



Compound	NAK	K_D (nM)	ΔH (cal/mol)	ΔS (cal/mol/deg)	N
AZD7762	AAK1	36.5	-1182	-7.0	1.04
	BIKE	33.1	-1485	-17.3	0.99
Baricitinib	AAK1	17.2	-1970	-33.0	1.05
	BIKE	39.8	-1541	-19.6	1.07
	GAK	134.4	-1136	-8.0	1.01
	MPSK1	68.5	-9731	-0.9	1.01
Nintedanib	AAK1	1060.4	-6320	5.4	1.03
	BIKE	104.2	-6255	10.2	1.05
AT9283	BIKE	6.0	-1056	1.0	1.07



As expected from sequence conservation, Inhibitor binding correlates best for AAK1 and BMP2K



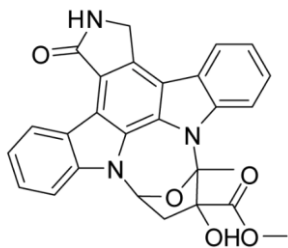
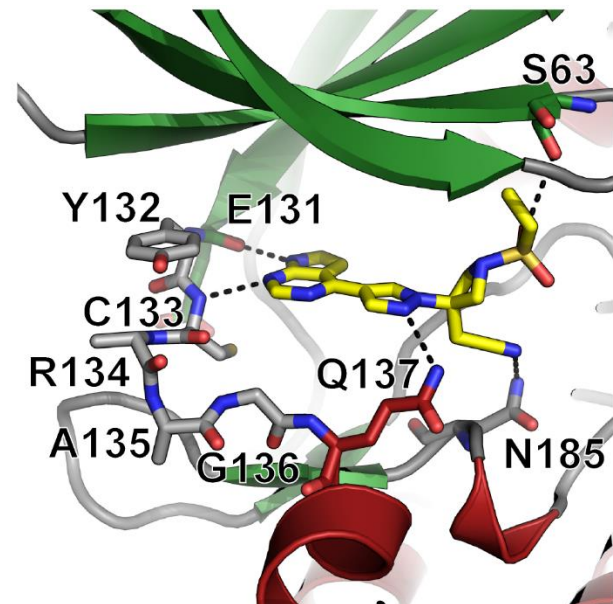
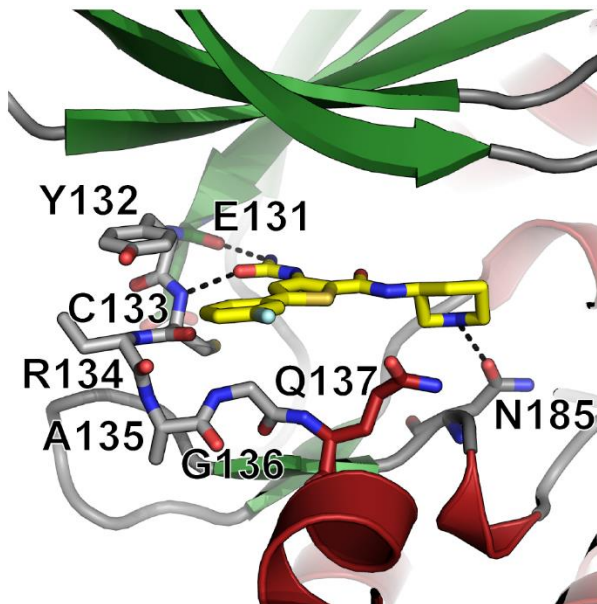
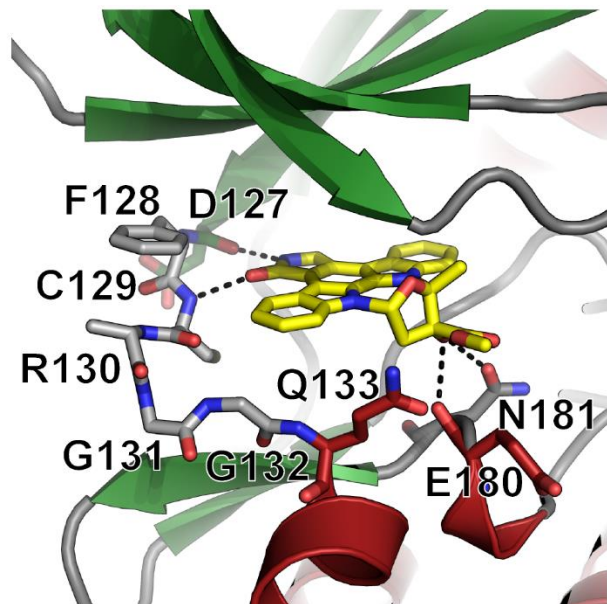
AAK1 / BMP2K co-crystal structures



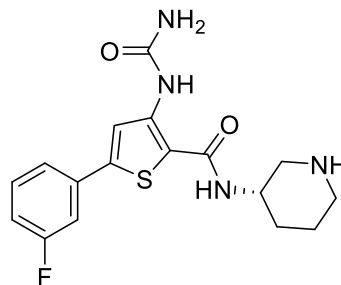
AAK1

BMP2K

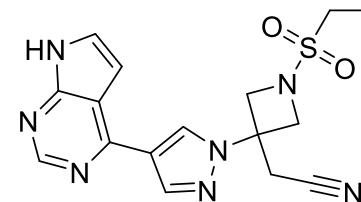
BMP2K



K252A

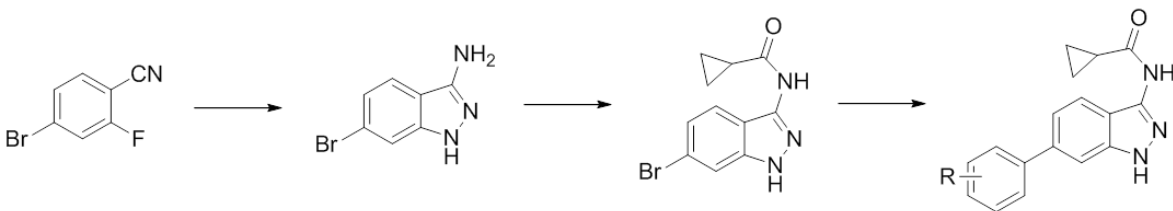


AZD7762



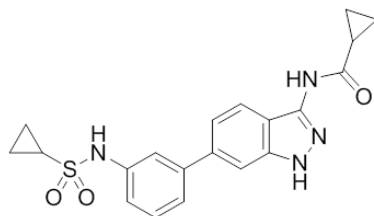
Baricitinib

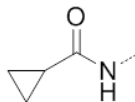
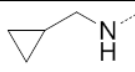
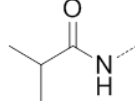
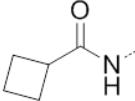
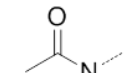
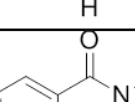
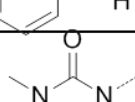
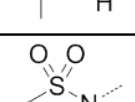
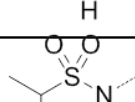
AAK1 probe SAR



R	AAK1 IC ₅₀ /nM
3-NHSO ₂ Me	220
4-NHSO ₂ Me	1200
3-CONH ₂	3800
4-CONH ₂	910
3-NHAc	1100
3-CO ₂ H	18000
4-CO ₂ H	2800
3-OH	350
4-OH	280
3-NH ₂	800

R	AAK1 IC ₅₀ /nM
3-NHSO ₂ Me	220
3-N(CH ₃)SO ₂ Me	120
3-CH ₂ NHSO ₂ Me	71
3-NHSO ₂ (isopropyl)	54
3-NHSO ₂ (cyclopropyl)	31

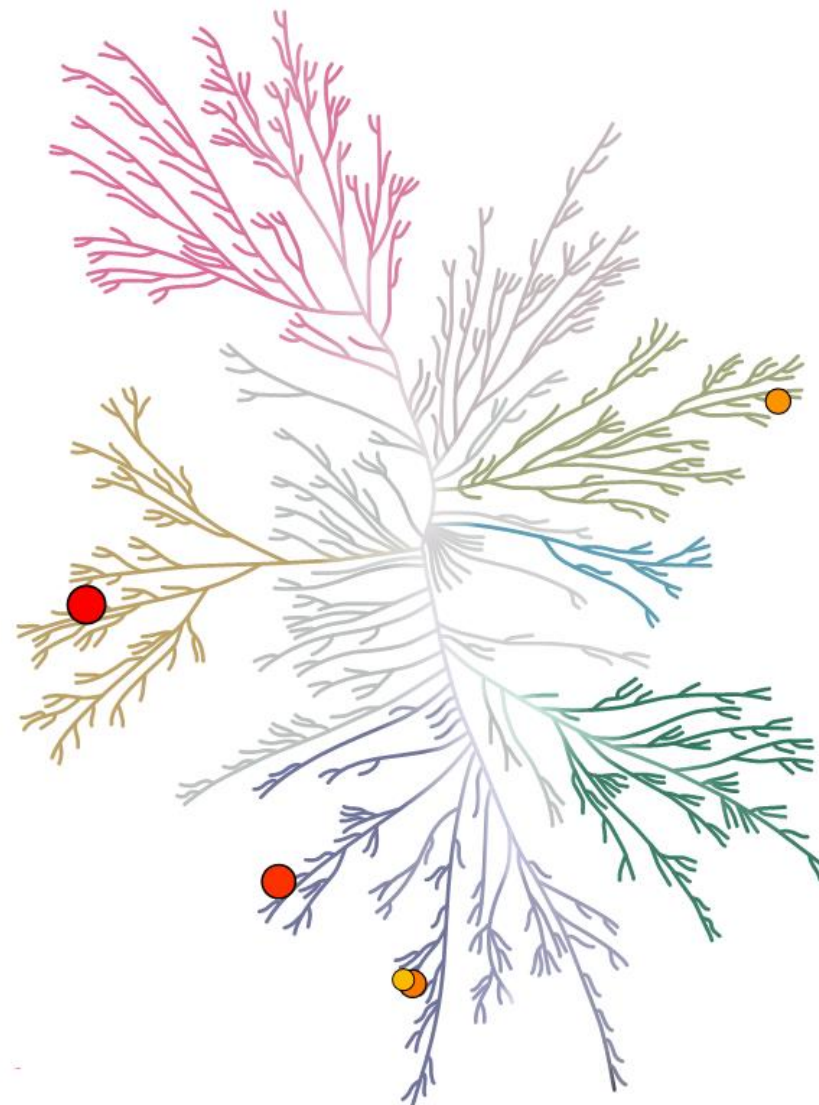
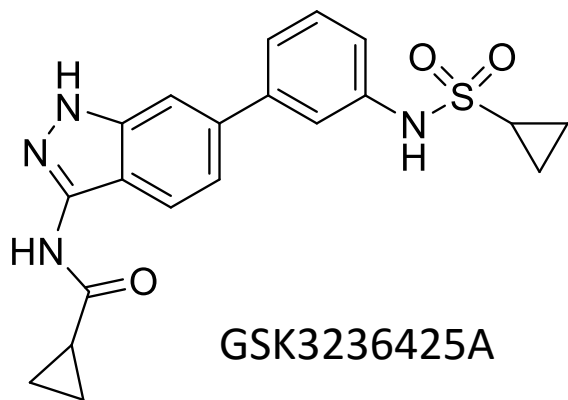


REG NO	R	AAK1 IC ₅₀ /nM
GSK3236425A		31
GSK3377198A		3500
GSK3236734A		18000
GSK3238095A		370
GSK3277335A		560
GSK3257224A		2200
GSK3369806A		> 50000
GSK3367090A		> 50000
GSK3367591A		7700

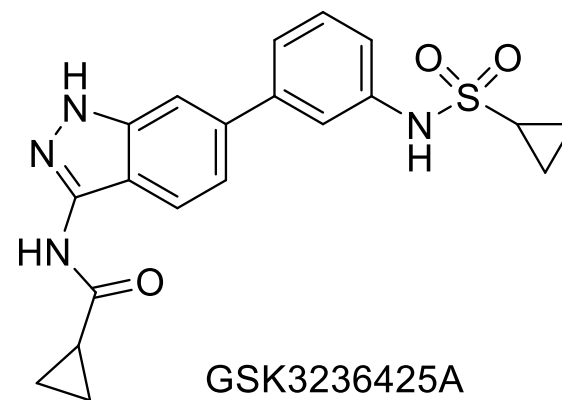
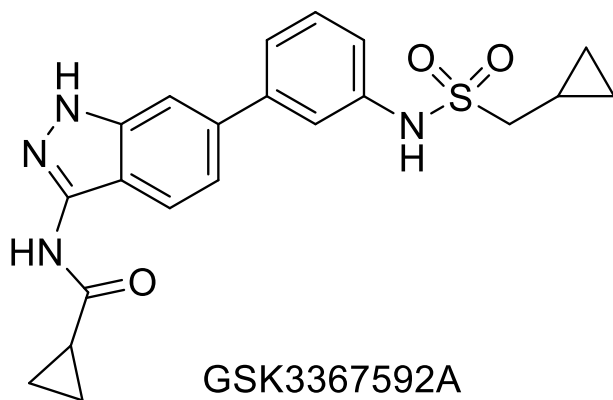
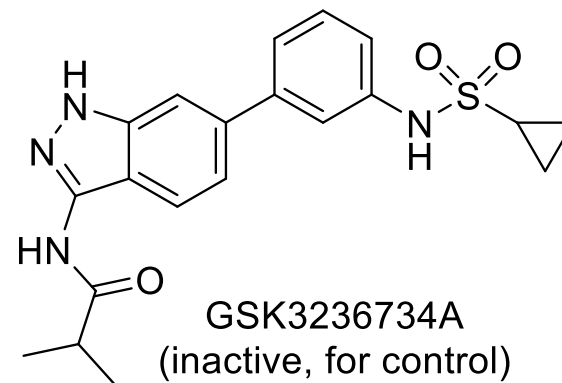
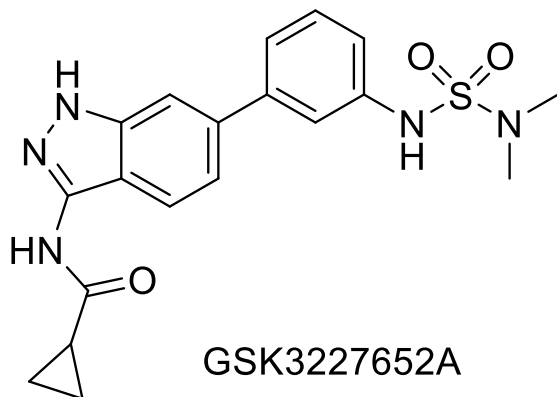
Reaction Biology Profiling 300 kinases:

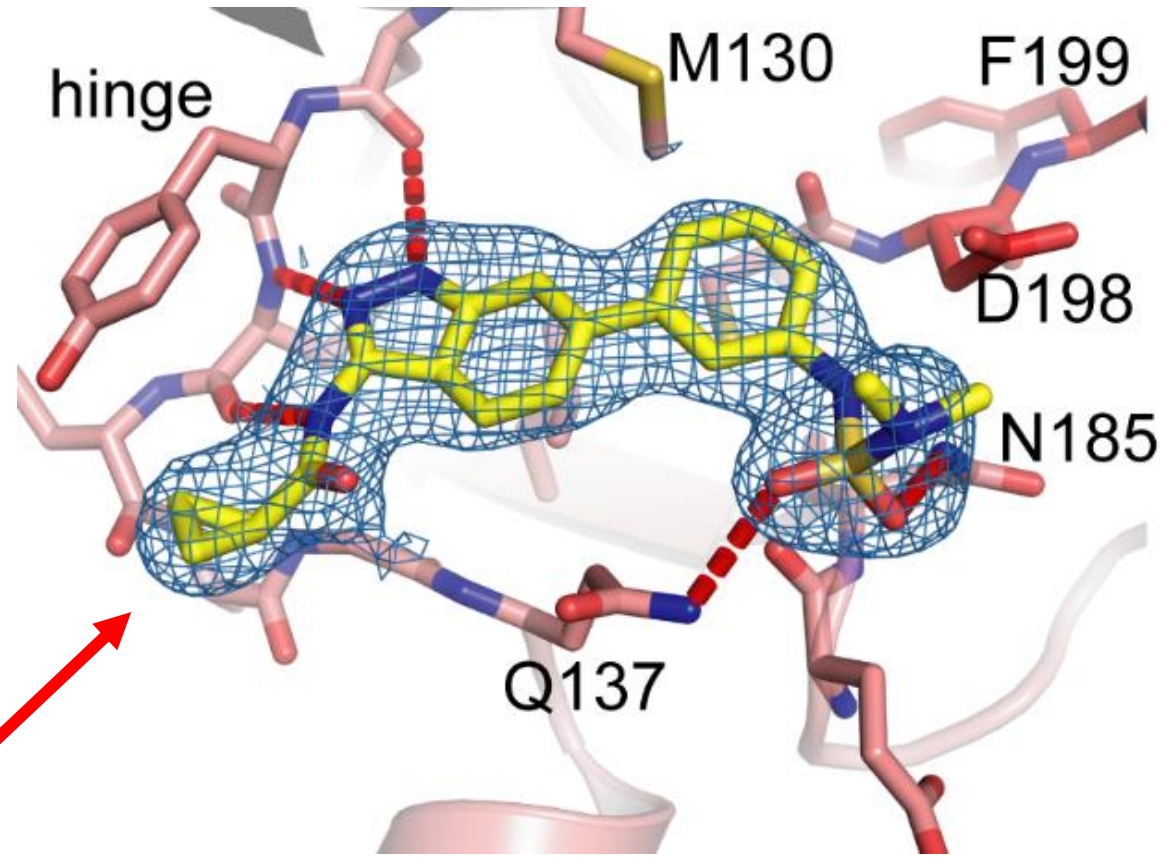
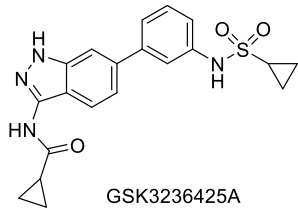
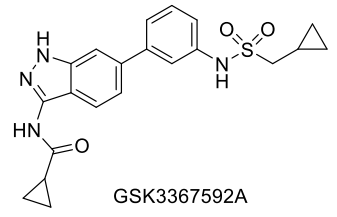
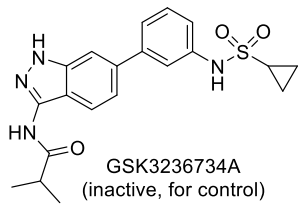
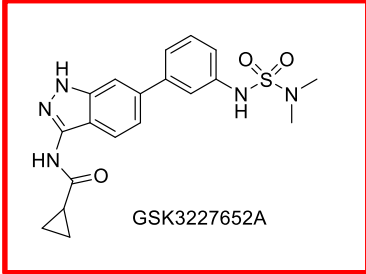
	% Remaining Activity (1 μ M GSK3236425A)
NLK	24
MLCK2/MYLK2	33
BRSK1	46
TNIK	51
BRSK2	58

(AAK1 & BMP2K not included in the panel)

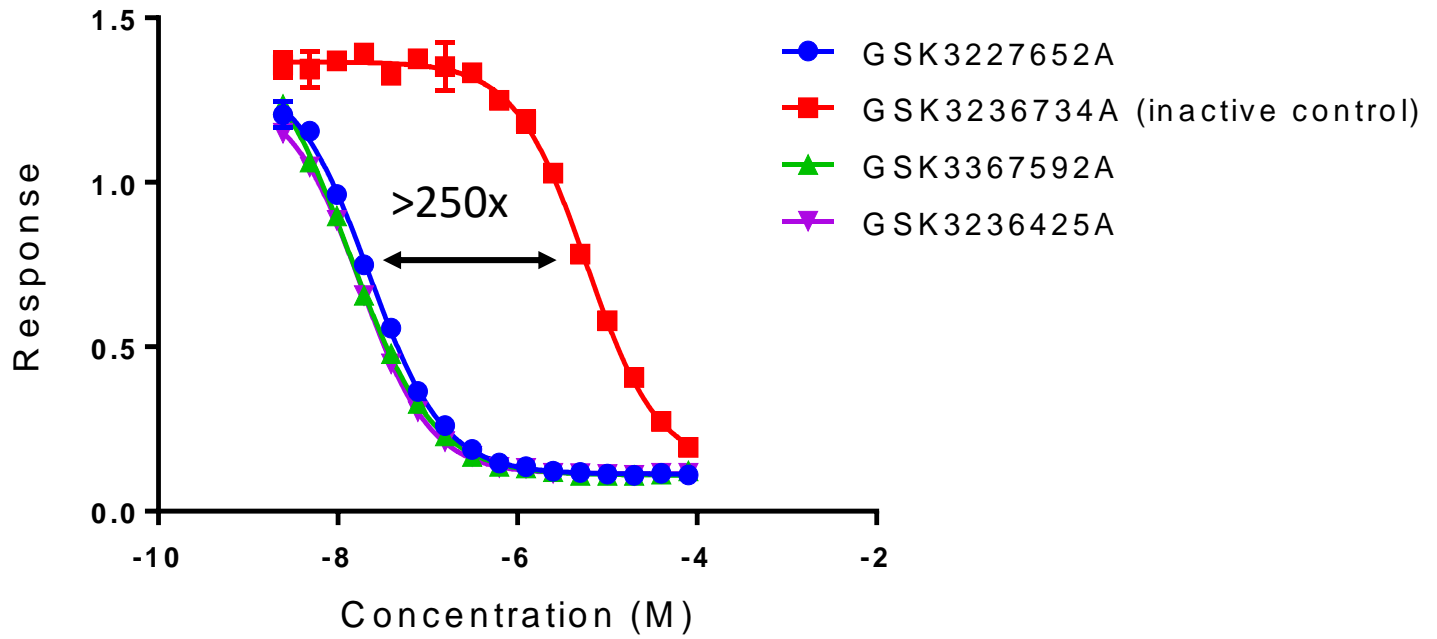


The series produced three inhibitors with IC_{50} (AAK1) \sim 30 nM





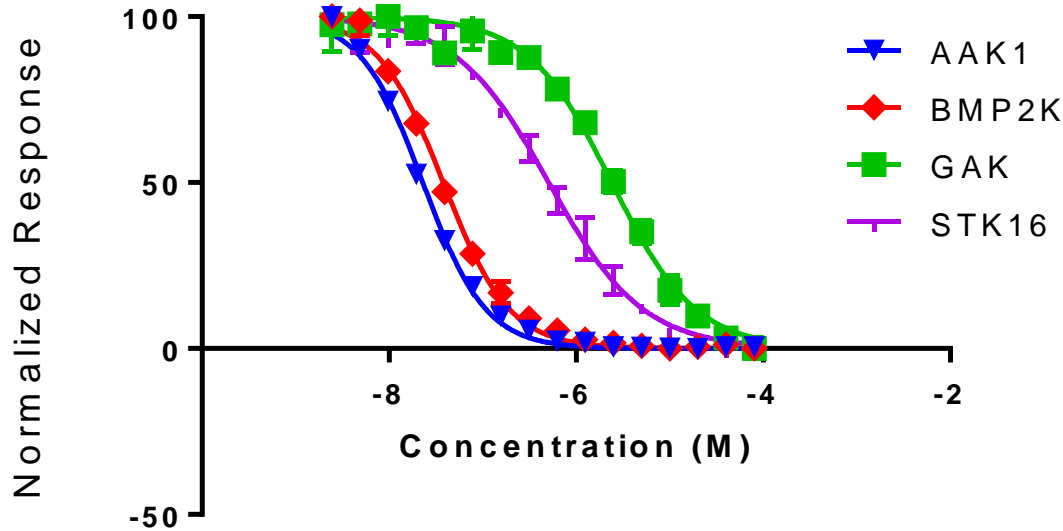
Why is cyclopropyl important?



Confirm selectivity



GSK3236425A

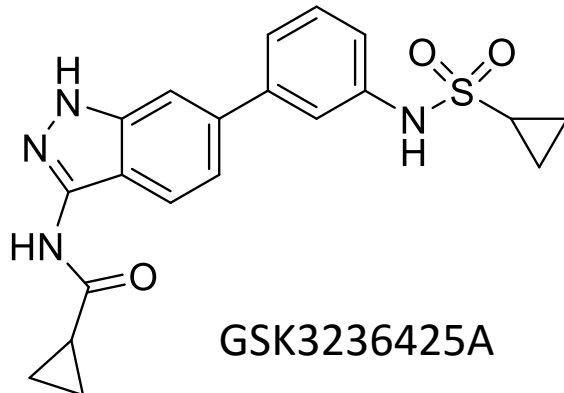


K_i values for selectivity analysis:

	nM
AAK1	8
BMP2K	13
GAK	1621
STK16	329

Fold selectivity:

AAK1	1x
BMP2K	1.6x
GAK	195x
STK16	40x



SGC Oxford

Fiona Sorrell
Marta Szklarz
Kamal R. Abdul Azeez
Pavel Savitsky
Stefan Knapp

SGC UNICAMP

Rafael Counago
Opher Gileadi

SGC UNC

Bill Zuercher
Carrow Wells
Alison Axtman
David Drewry
Tim Willson

About the SGC

The SGC is an international public-private partnership (UK charity number 1097737) that aims to carry out basic science of relevance to drug discovery, placing all information, reagents and know-how into the public domain without restriction.

jon.elkins@sgc.ox.ac.uk

Tel: +44 (0) 1865 617751

Structural Genomics Consortium
Old Road Campus Research Building
Roosevelt Drive
Oxford
OX3 7DQ
U.K.