Targeting Phosphorylation Signalling Networks

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SGC Toronto

SGC Oxford

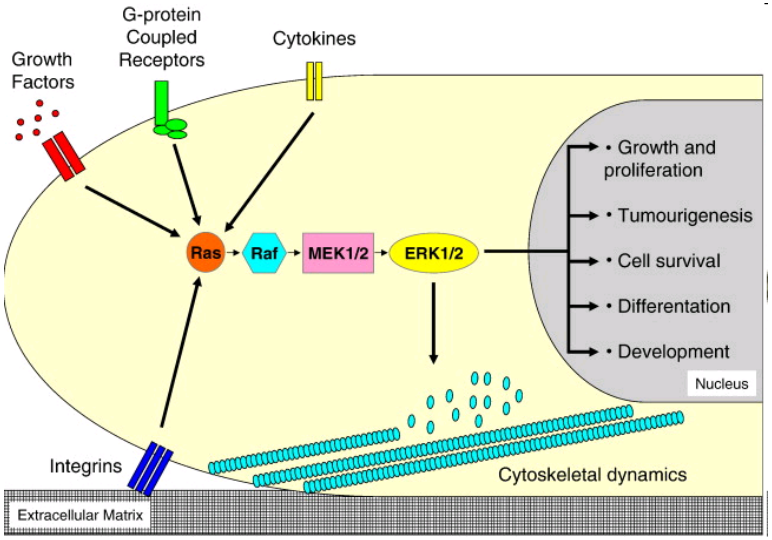
SGC Stockholm

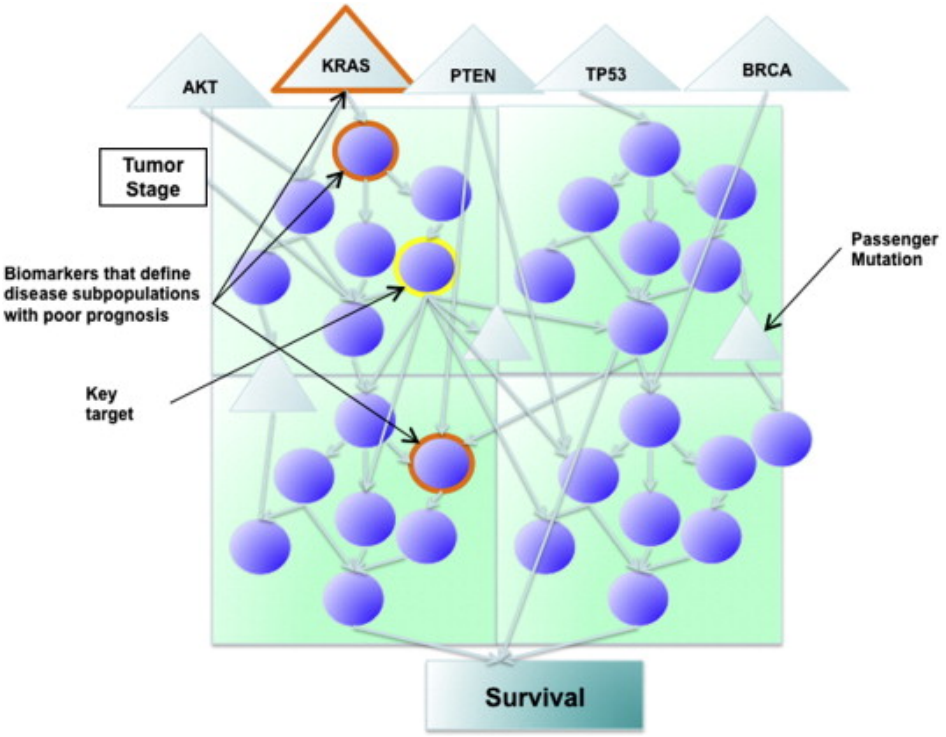
Complexity of Cellular Signalling

Limited understanding of cellular signalling

- Large number of mutations in tumours
- Best entry point of pharmacological intervention is not known
- Detection of an oncogenic kinase mutation does not guarantee sensitivity to inhibition

Cascades **vs** **Networks**

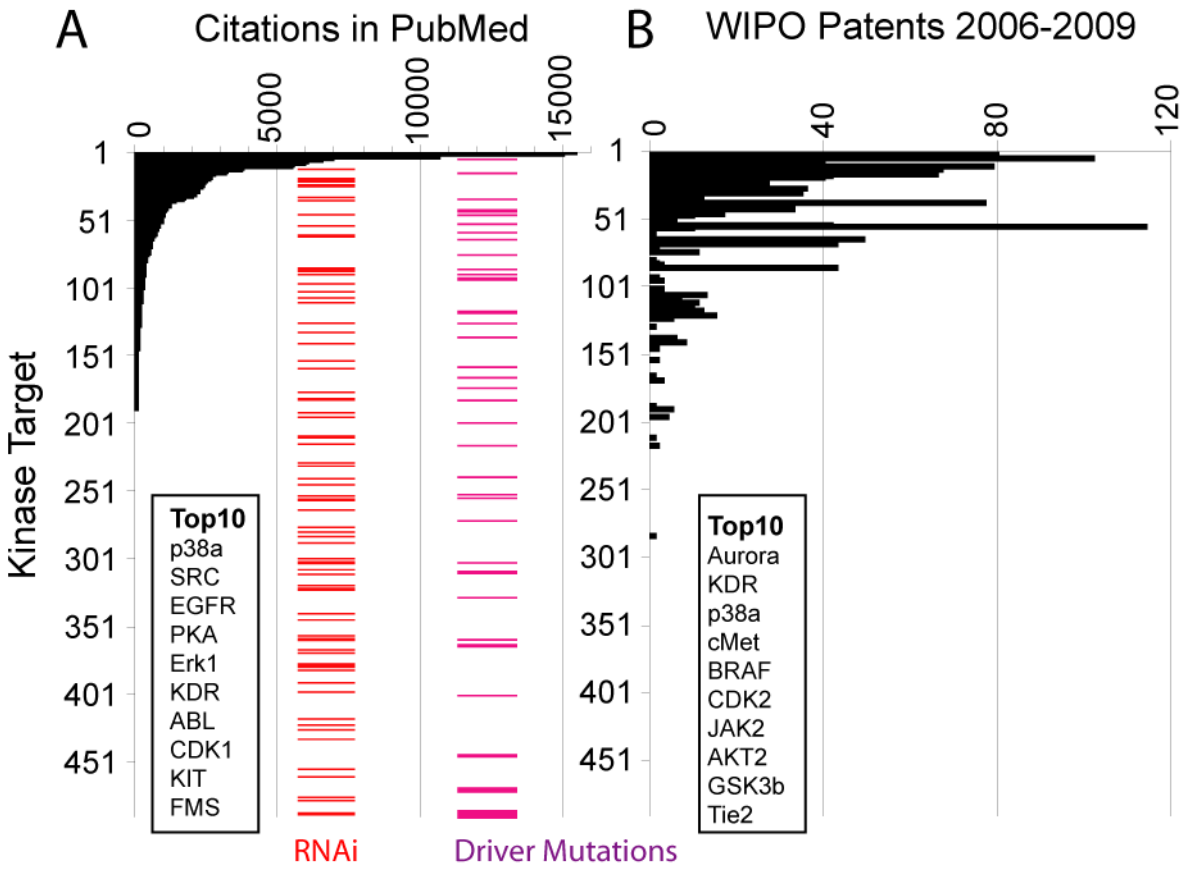


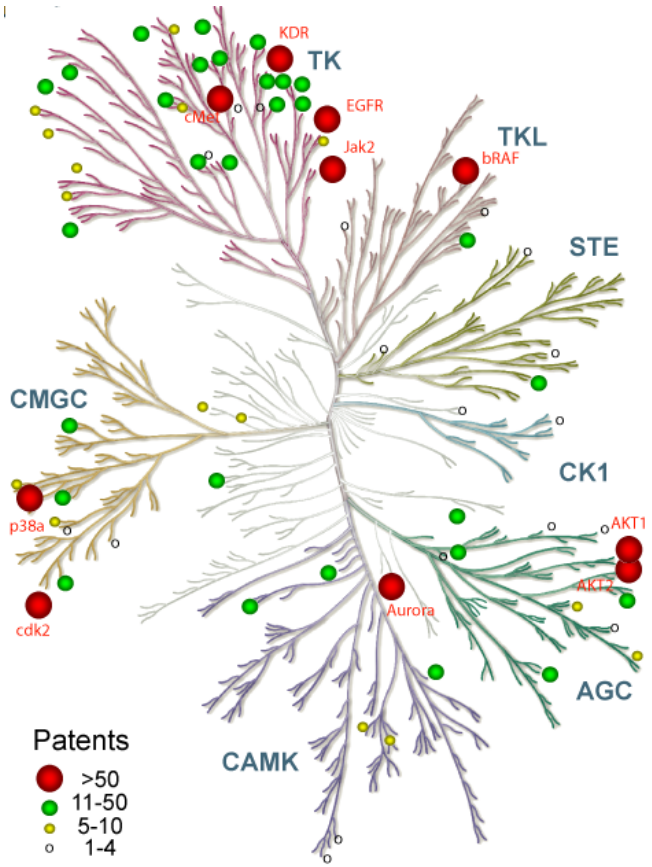


(Un)-explored Kinome – what we know about the Network

Covering mainly ~10% Kinome
Patents follow public data

Kinases: > 500 000 papers in PubMed
> 10 000 US patents





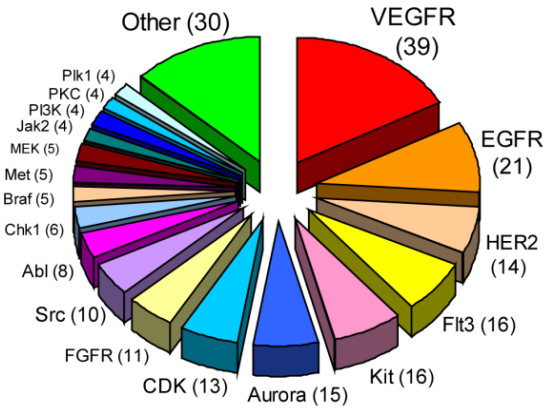
C

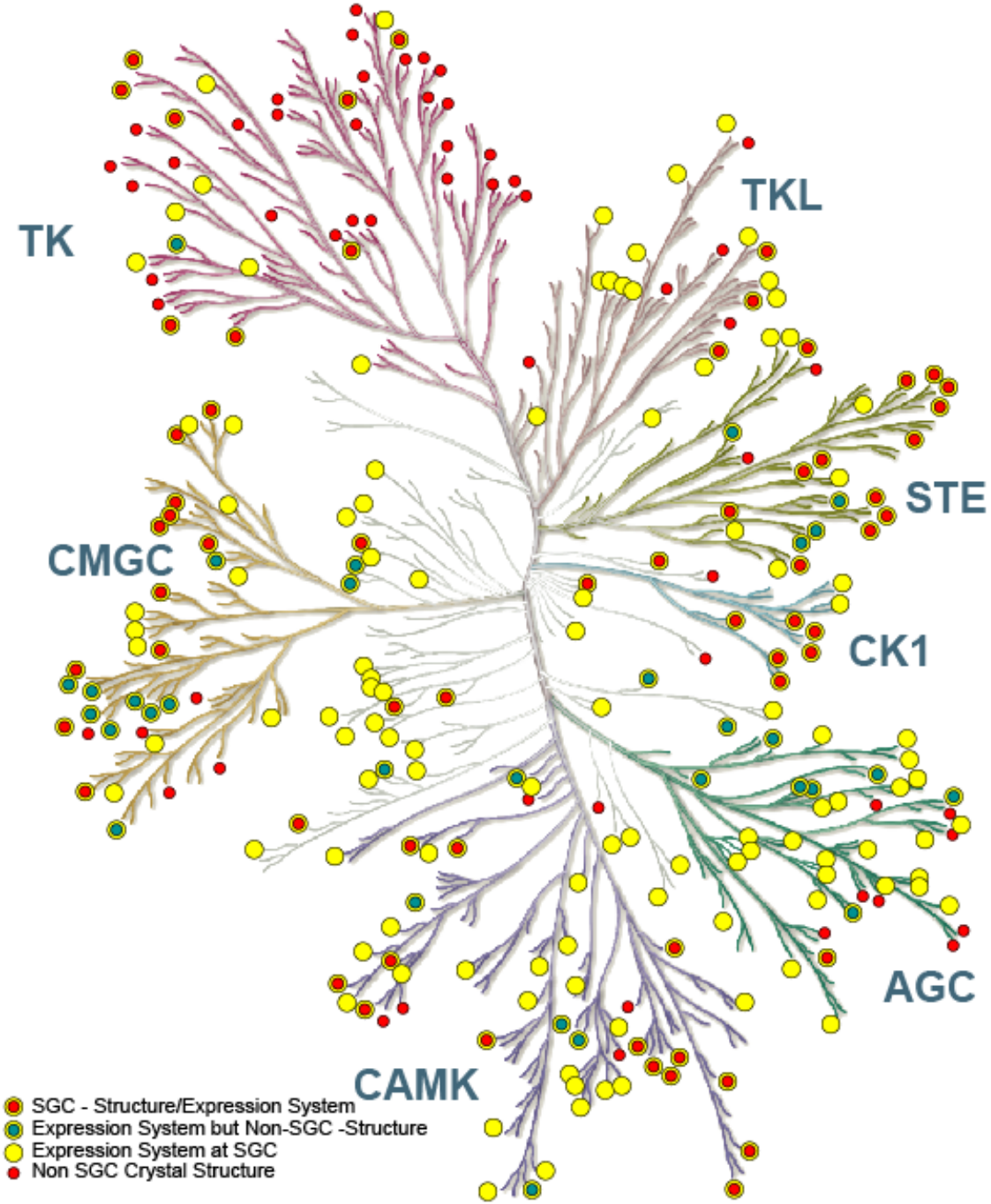
Limited understanding

- Large number of mutations
- Best entry point of pharmac
- Detection of an oncogenic k inhibition

Clinical POC is main m

> 50% of clinical inhibitors t already approved





Overcoming Selectivity Problems ?

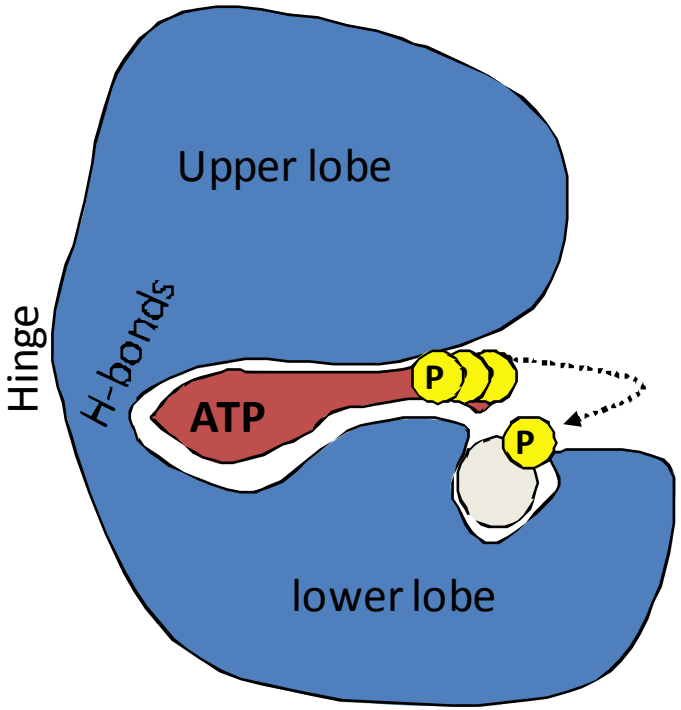
SBDD strategies

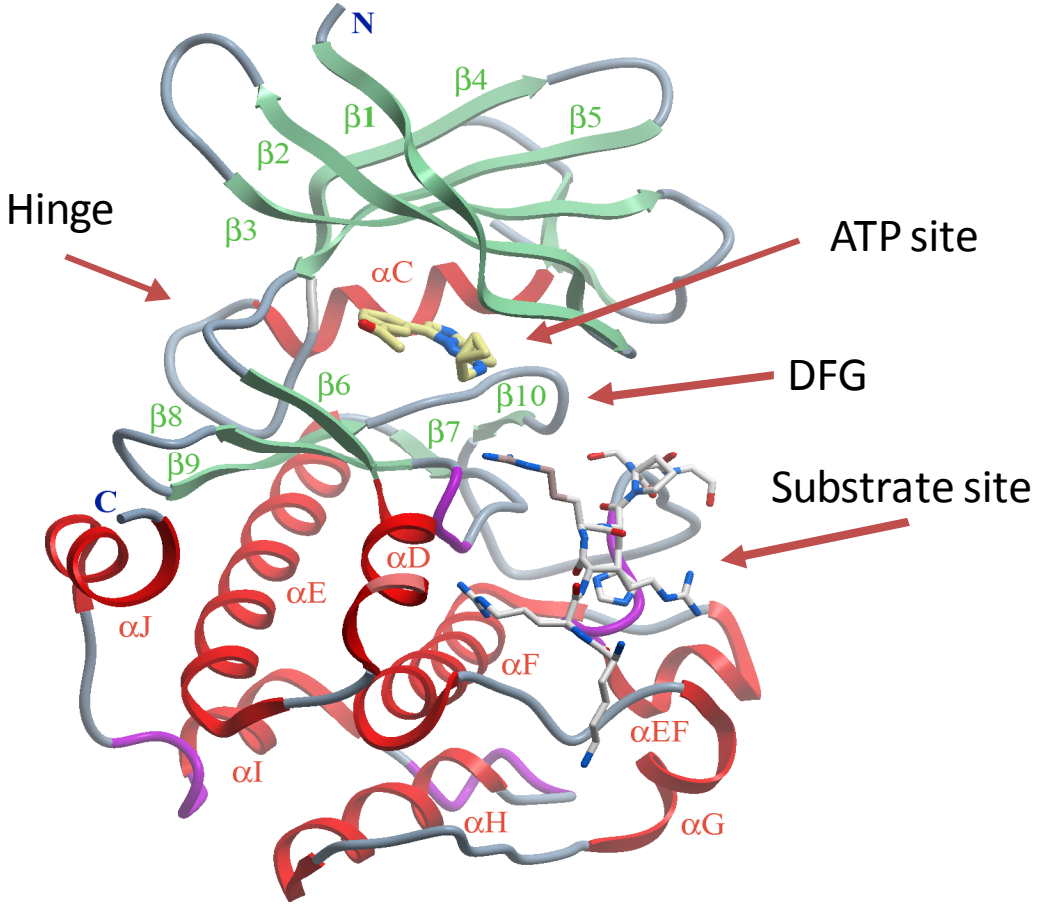
- **Out of the "Box" inhibitors (Allosteric Inhibitors, Reg. Domains)**
- **Targeting inactive conformations (DFG out)**
- **Targeting unique kinases**
- **Targeting unique binding modes**
- **Targeting unique active site features**

Requirements:

- Complex with inhibitor scaffolds with target and cross reacting kinases
- Sufficiently large (representative) screening panel

Targeting the Active Site





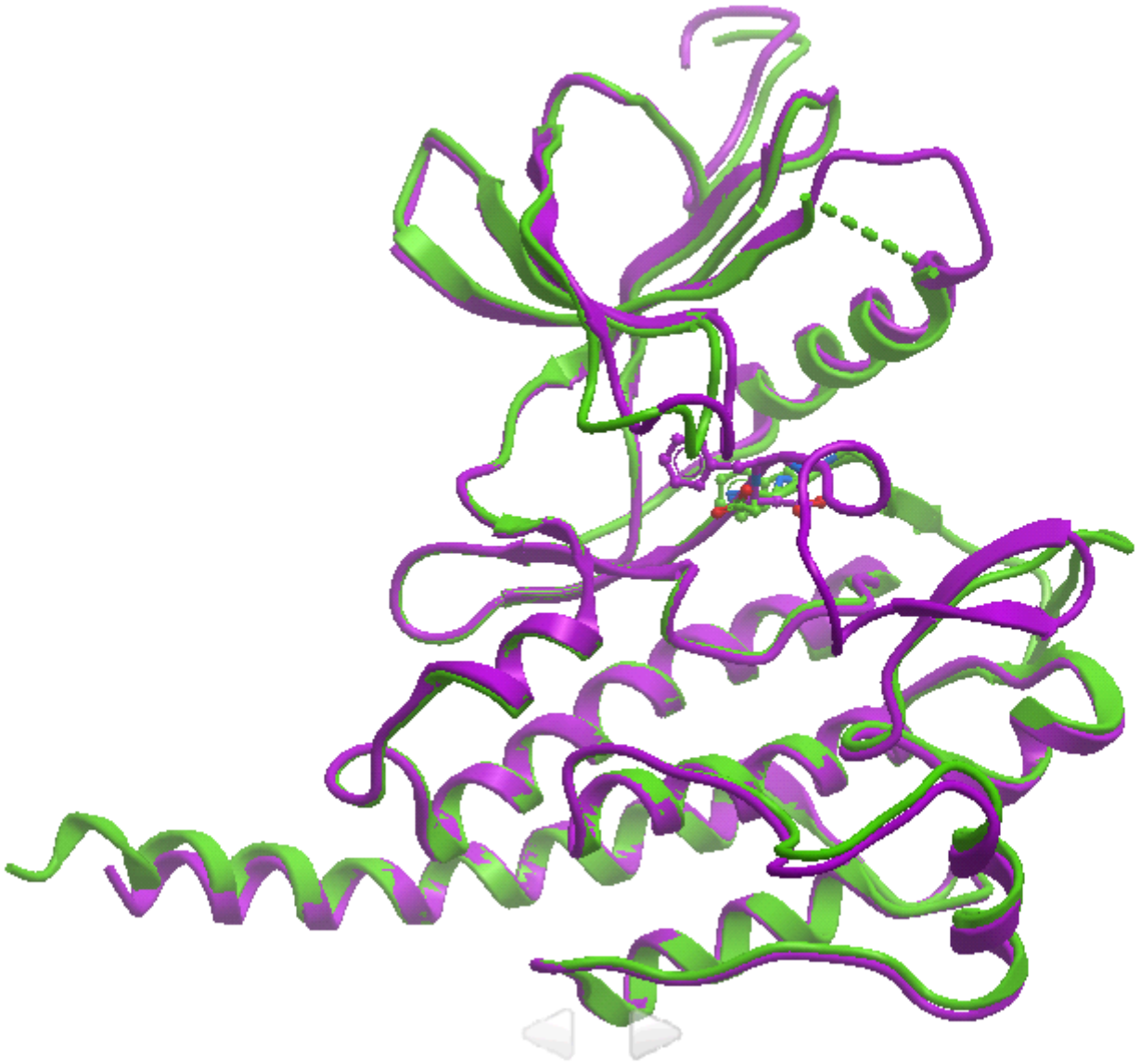
Kinase Inhibitors

ATP mimetic: type I

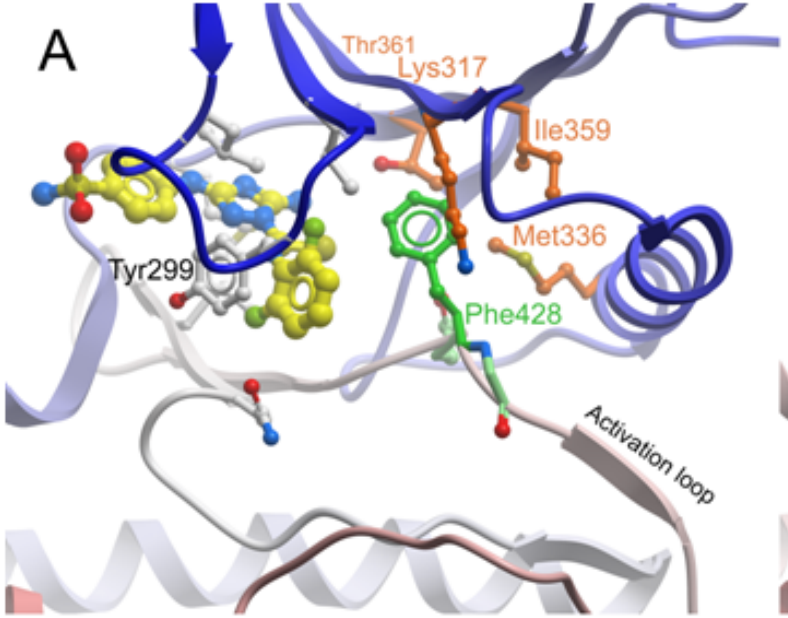
ATP competitive binding inactive conformation: type II

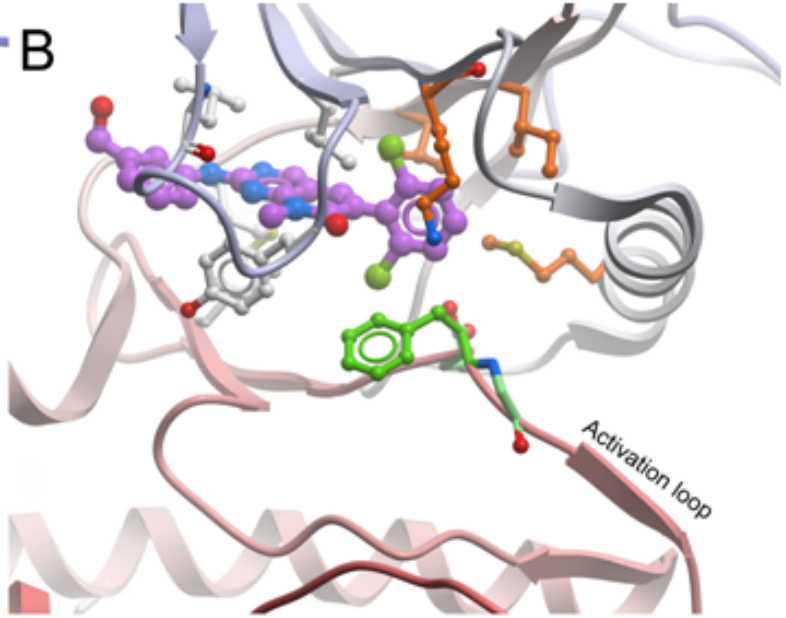
Substrate competitive: type III

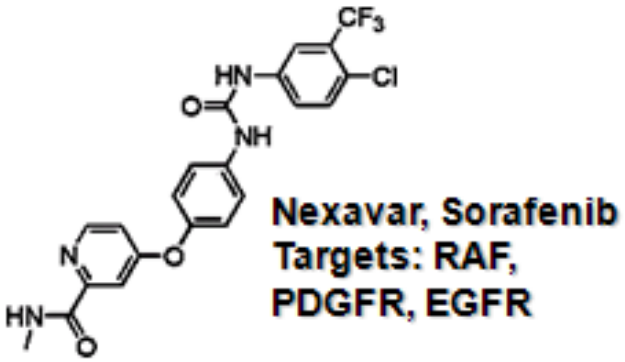
Inhibitors Types (Type I/II)



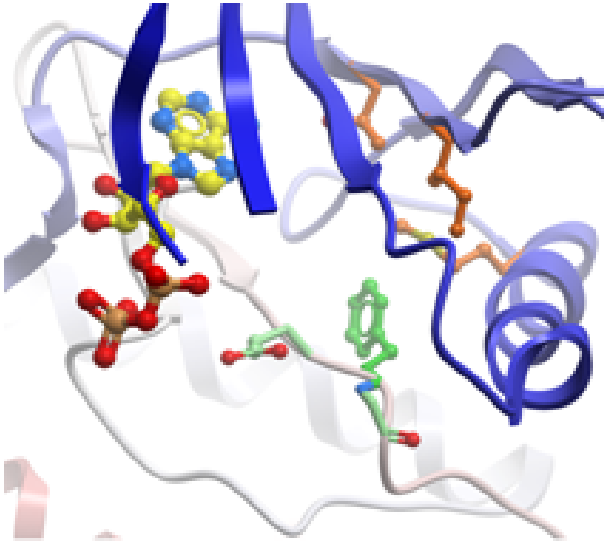
Targeting Intermediate Conformations





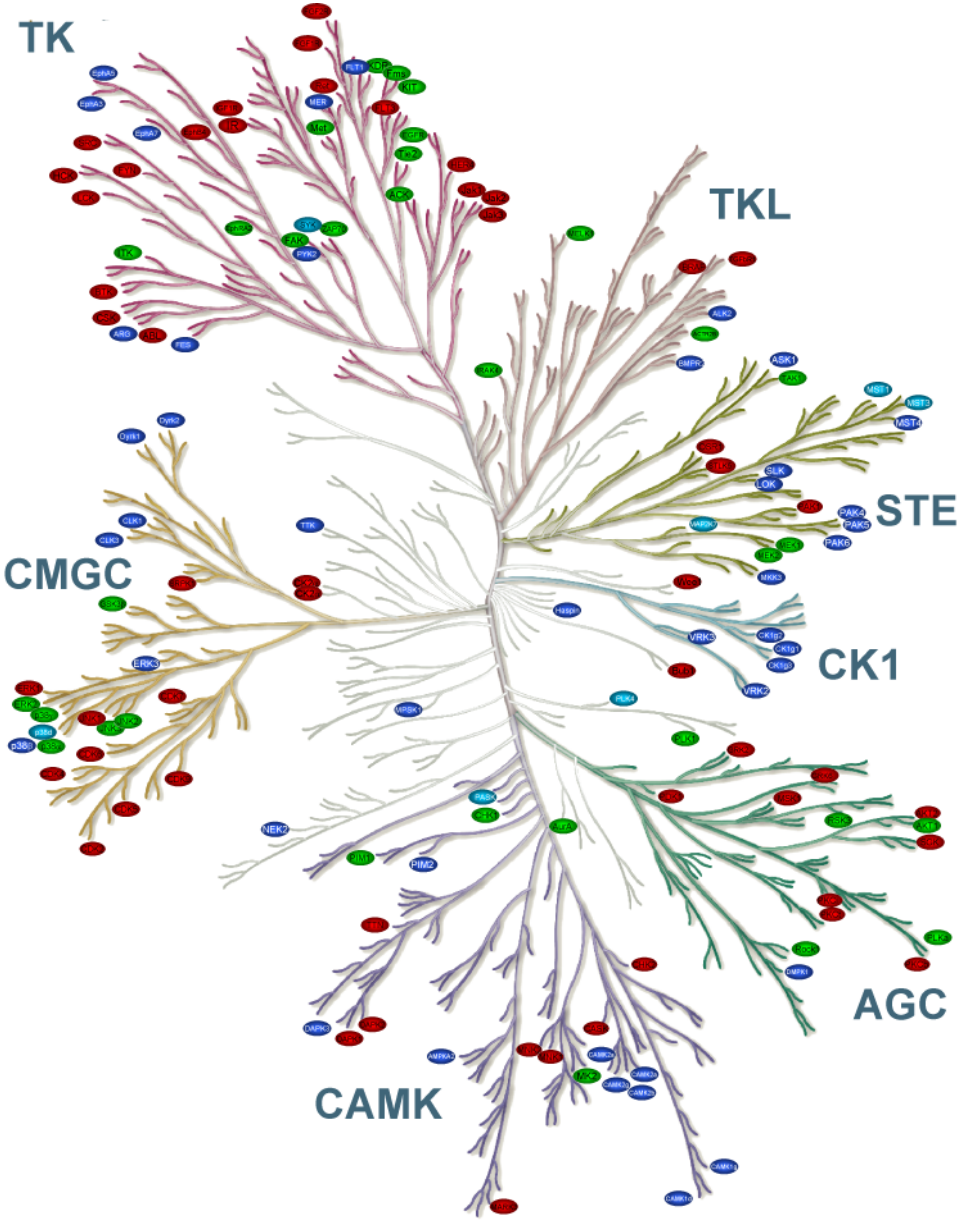


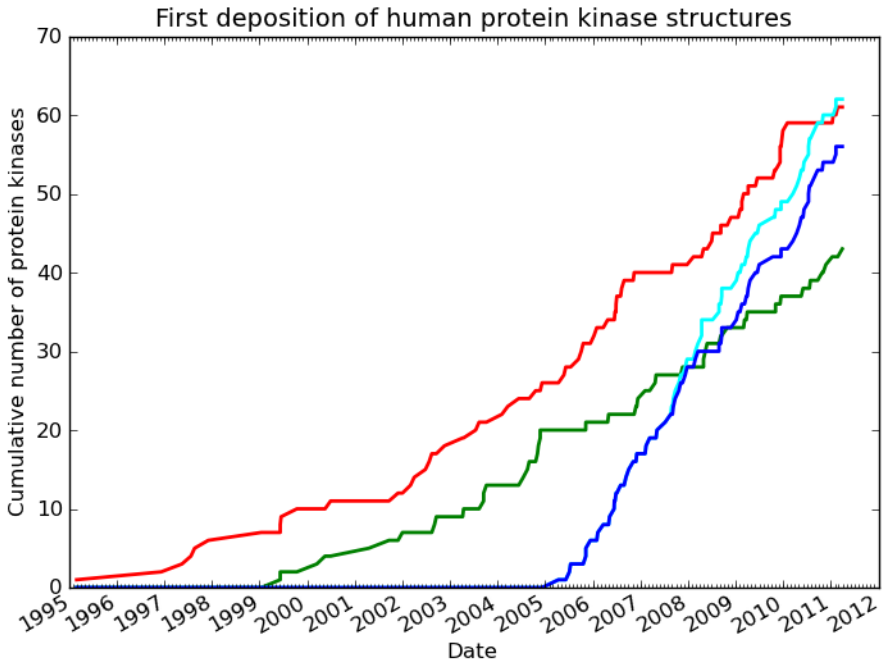
Nexavar, Sorafenib
Targets: RAF, PDGFR, EGFR



ABL1 : ADP (active, DFG in)

Kinase Family Wide Structural Analysis

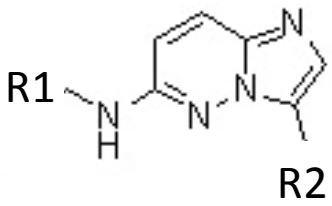




- 61 Structure in PDB from Academia
- 42 Structures in PDB from Industry

56 Human Kinase Structures by SGC since 2004

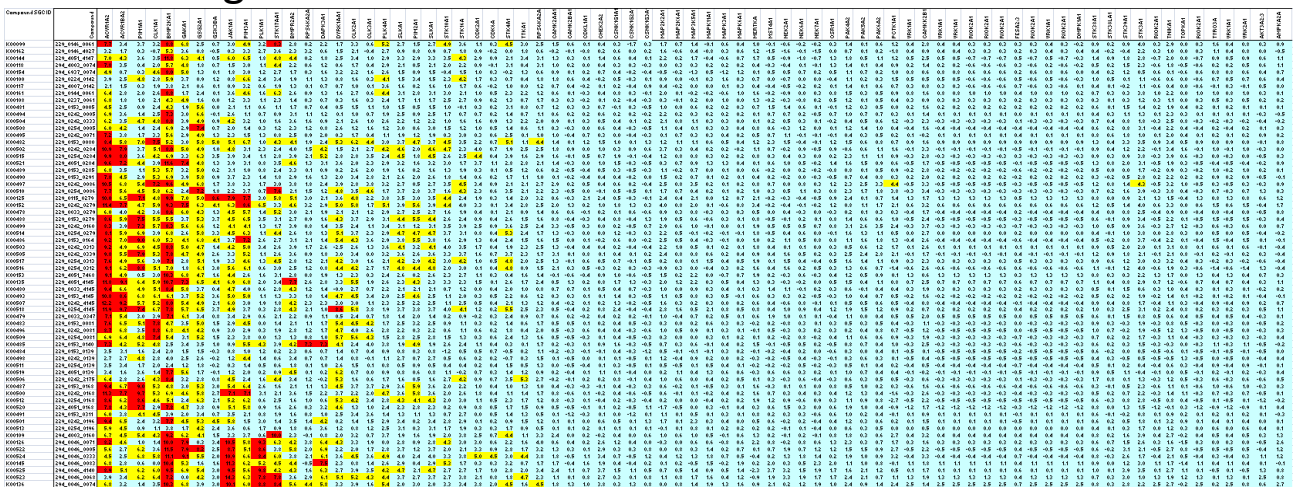
Early Parallel Screening

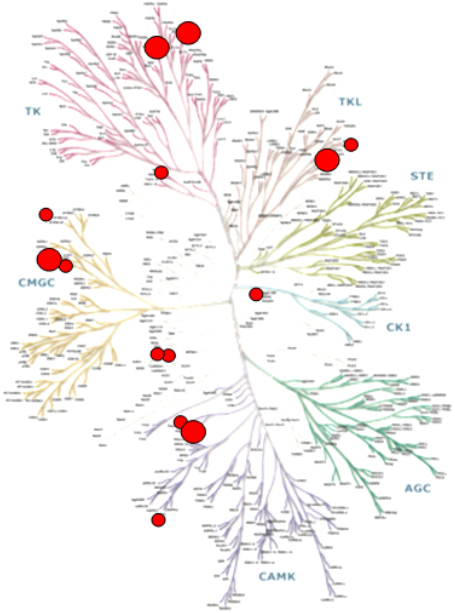


imidazo[1,2-*b*]pyridazine 1

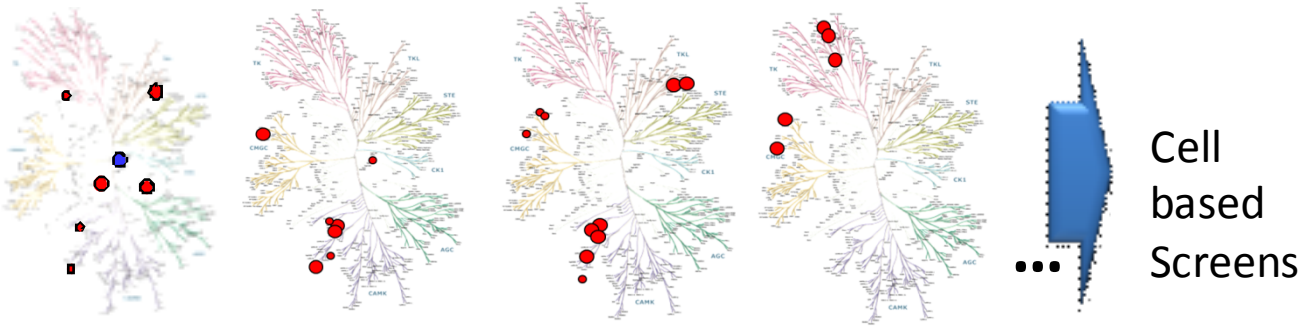
Kinase Target

Inhibitor





Subset of Kinases that can be targeted with tested scaffold
ID cross reacting kinases early

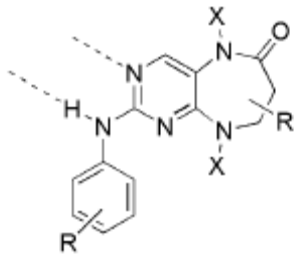


Cell based Screens

~300 kinases screened

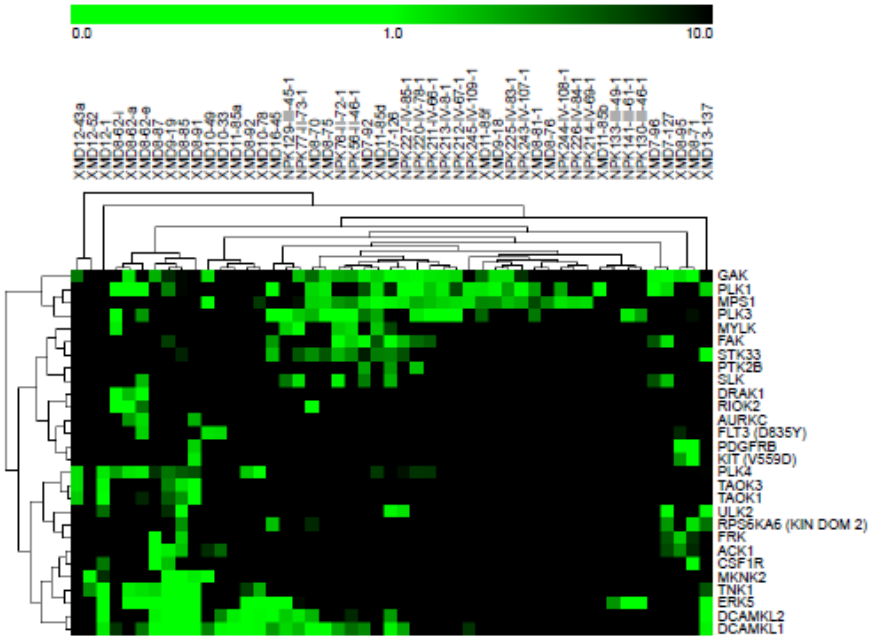
Chemical array of well characterized inhibitor selectivity

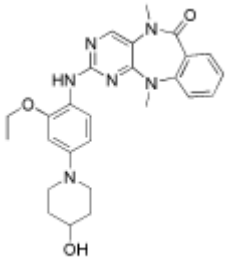
Parallel Screening of KTLs



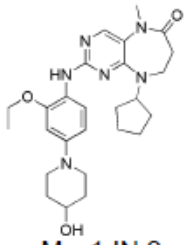
X= H or Me

Pyrimido-diazepines (~60 compounds made and screened against AMBIT panel (~350 kinases)

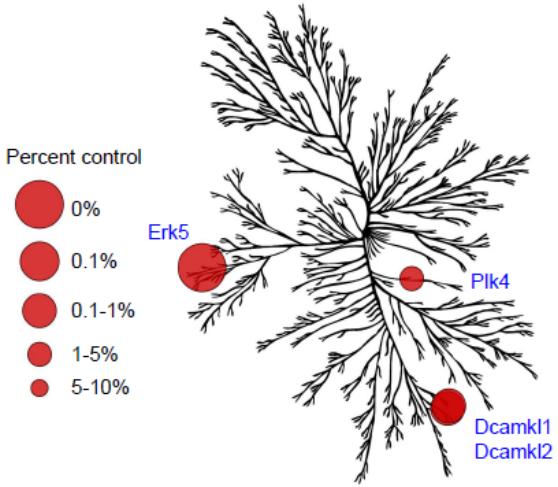




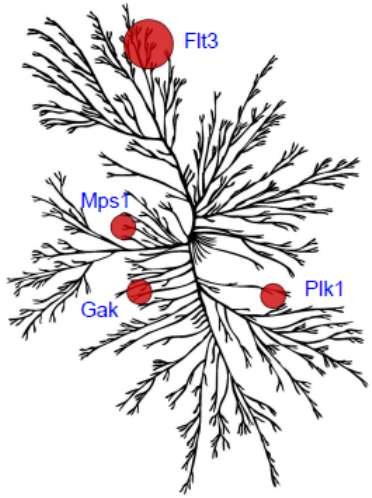
XMD8-92



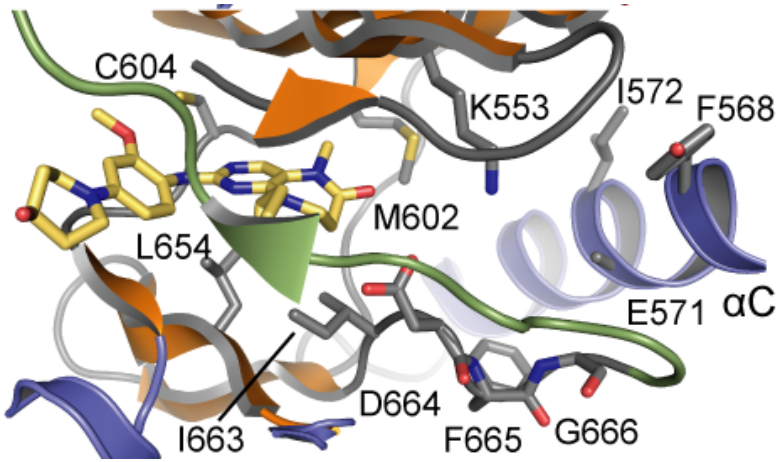
Mps1-IN-2



S(10)= 0.014
K_d (Erk5)= 80 nM

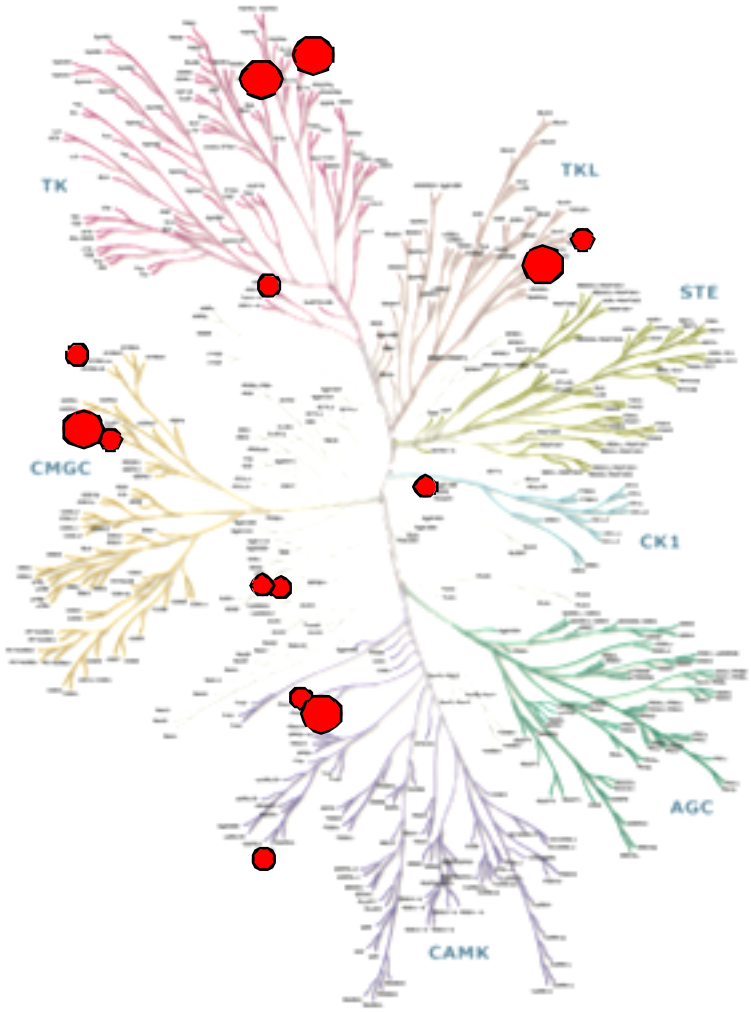


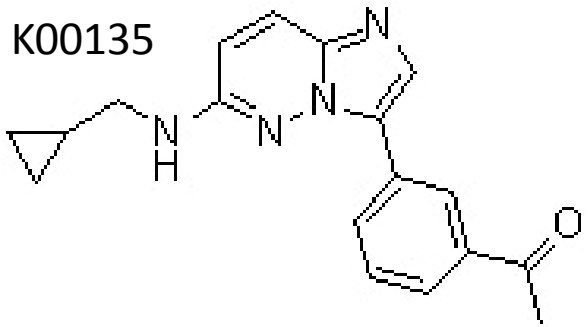
S(10)= 0.011
K_d (Mps1)= 26 nM



Collaboration with Gray Lab, Dana Faber, Harvard

Mechanisms of Cross-Reactivity





imidazo[1,2-*b*]pyridazine 1

<u>Originally identified as PIM1 inhibitor (hit from a purchased library / Biofocus)</u>

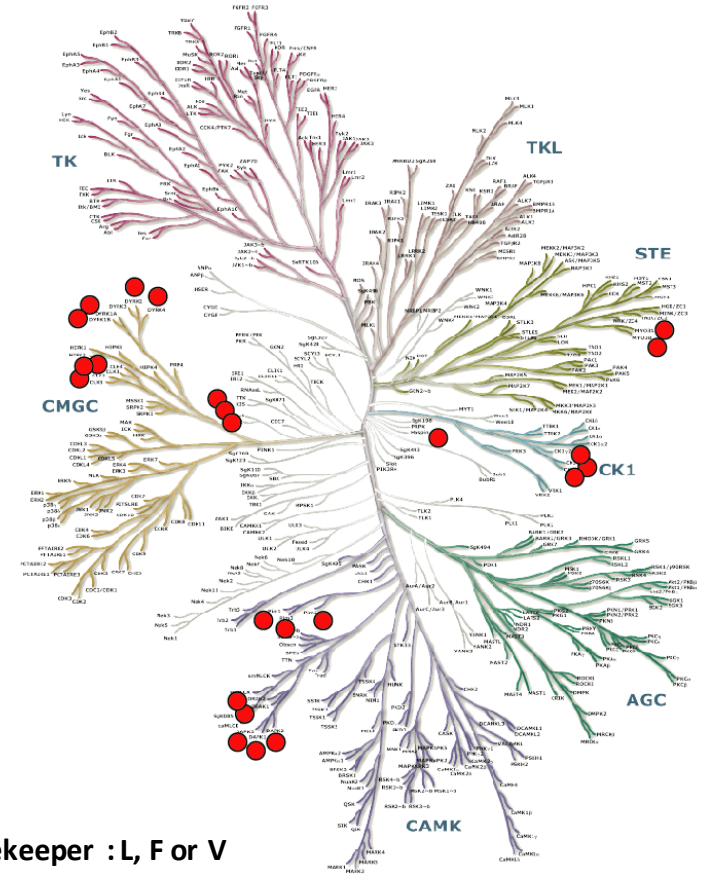
<u>Main cross reactivity</u>:
ACVR1, BMPR1, CLK1, BIKE, GAK, Haspin
KIT, FLT, DYRK1

- Selectivity for closely related isoforms (e.g. Pim1 vs Pim2; Clk1 vs Clk3)

- Cross reactivity with diverse kinases

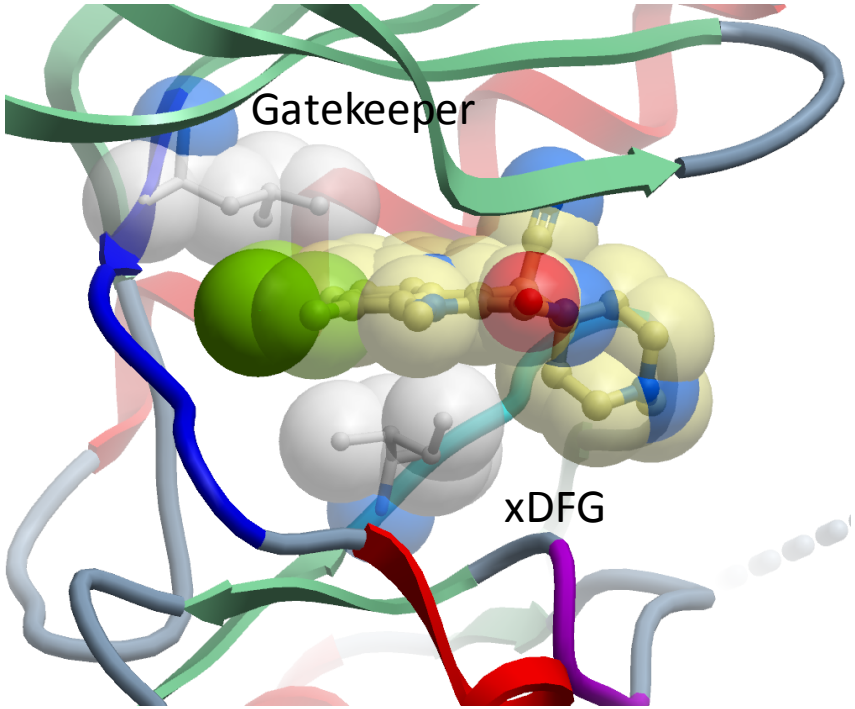
Screening data ~300 kinases and 180 in ΔTm assay

Unique Active Site Features



Gatekeeper : L, F or V
xDFG : V, I or L

Binding relies on 2 hydrophobic anchors

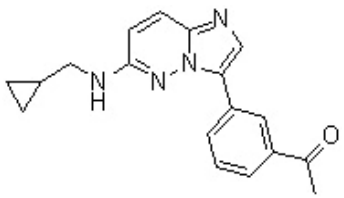


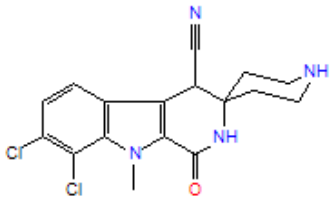
Scaffold binds to two hydrophobic anchor points

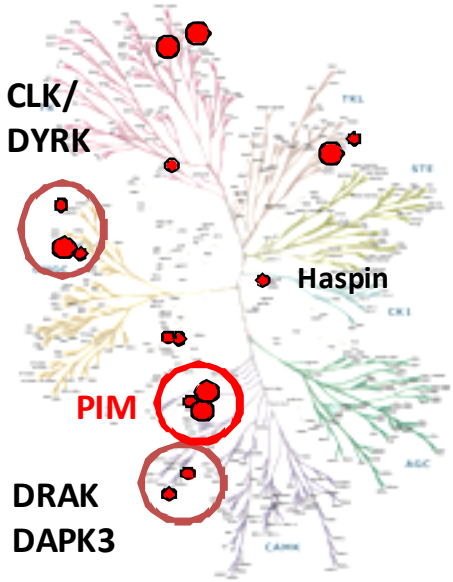
- **xDFG is rarely a large hydrophobic residue**

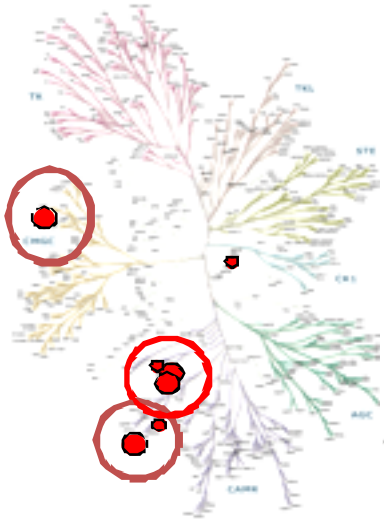
- **No classical hinge H-bond interaction**
- **ATP sites that contain xDFG anchor are very diverse**
- **Some bulky ligands will not fit into all sites**

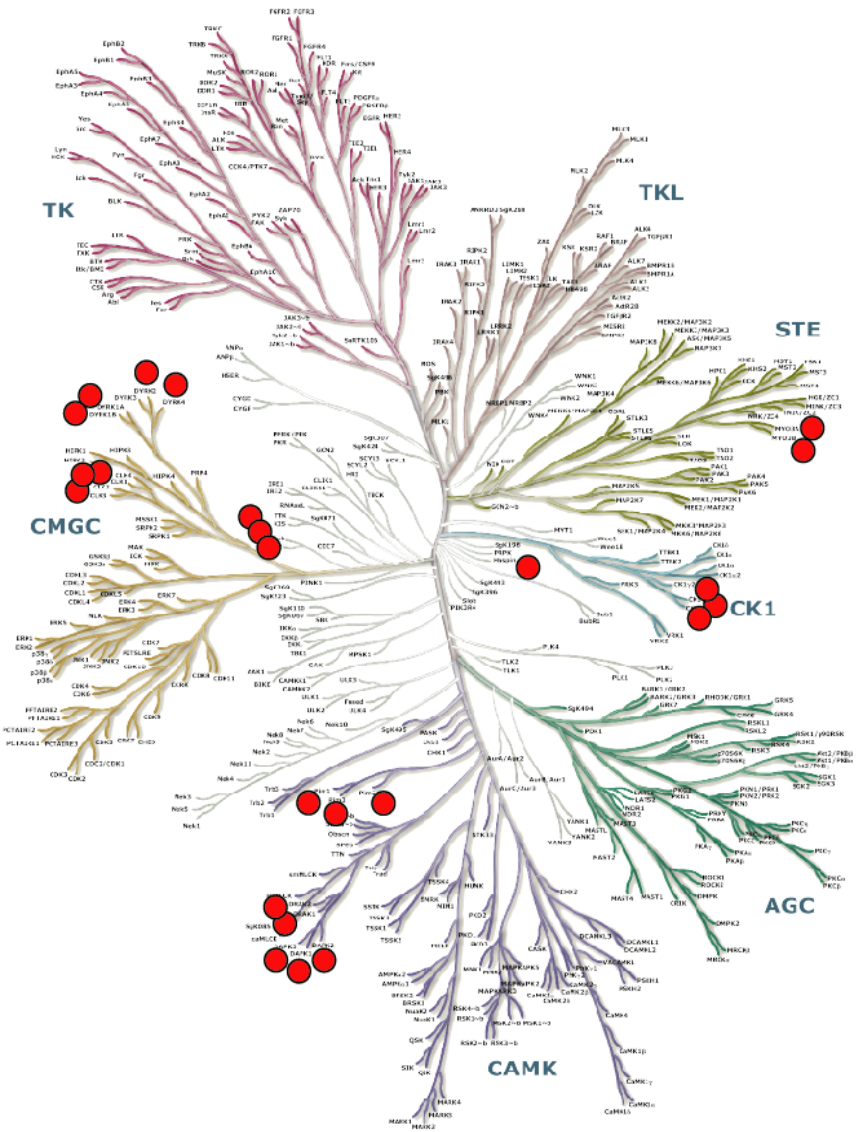
Cross-Reactivity – Non ATP mimetic









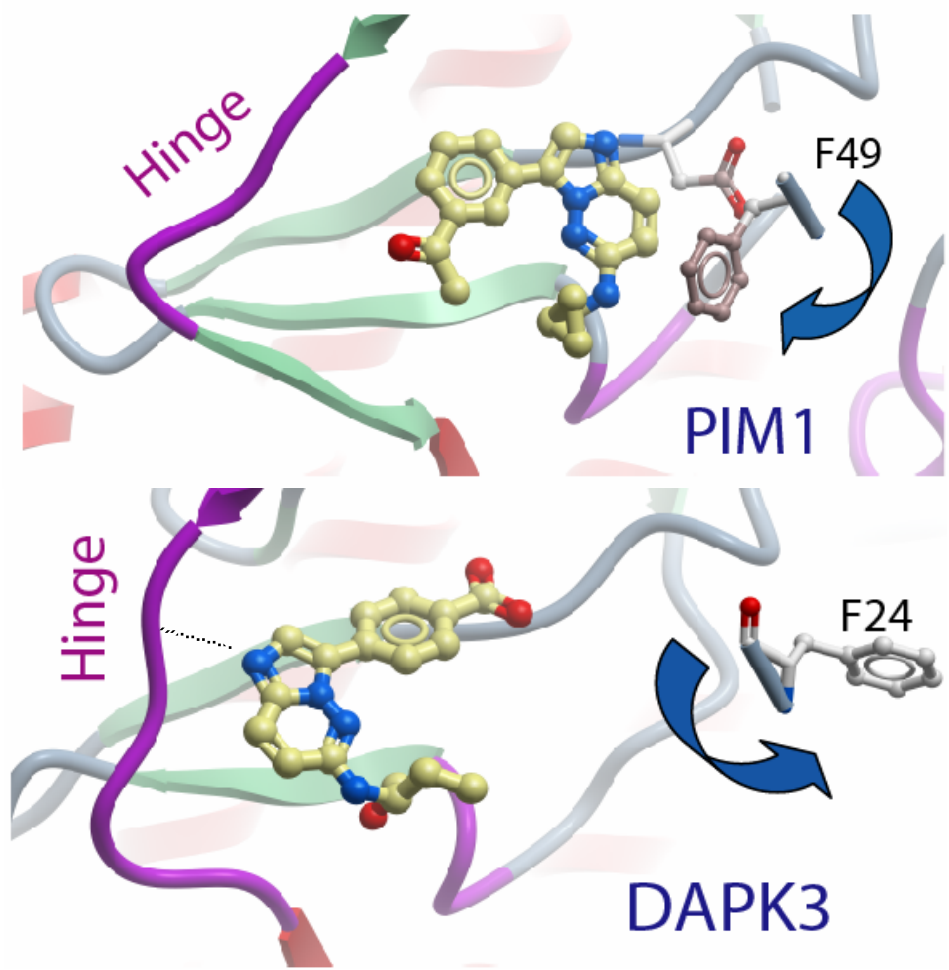


ΔTm > 6 °C
ACVR1, PIM1/3
BMPR1, CLK1, KIT, FLT
ΔTm > 4 °C
BIKE, GAK, Haspin, PIM2
DRAK1, DAPK3, DYRK1/2

ΔTm > 6 °C
PIM1/3, CLK1, DAPK3,
ΔTm > 4 °C
Haspin, DRAK1, DYRK1/2

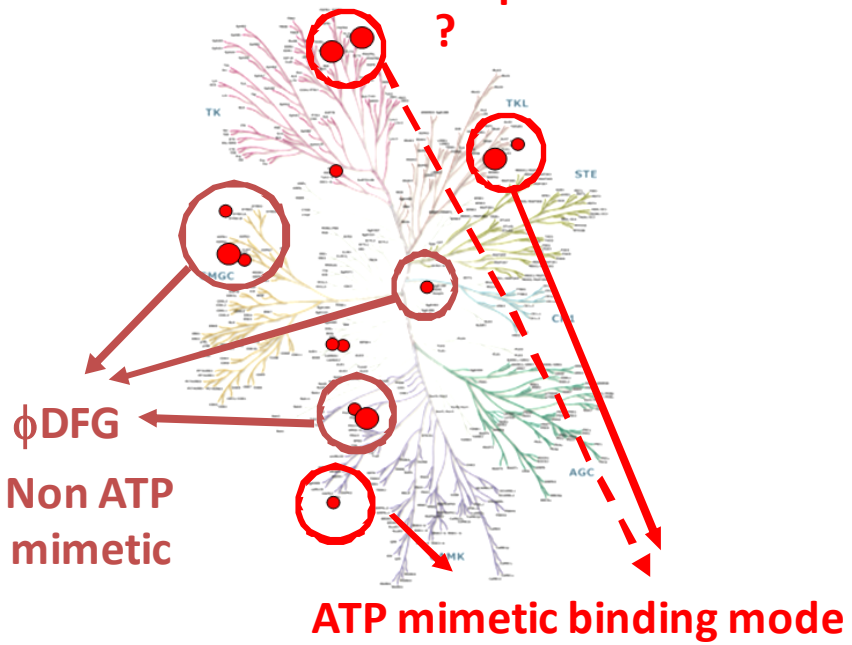
Mechanisms of Cross-Reactivity

Imidazo-pyridazines change orientation when binding to active kinases

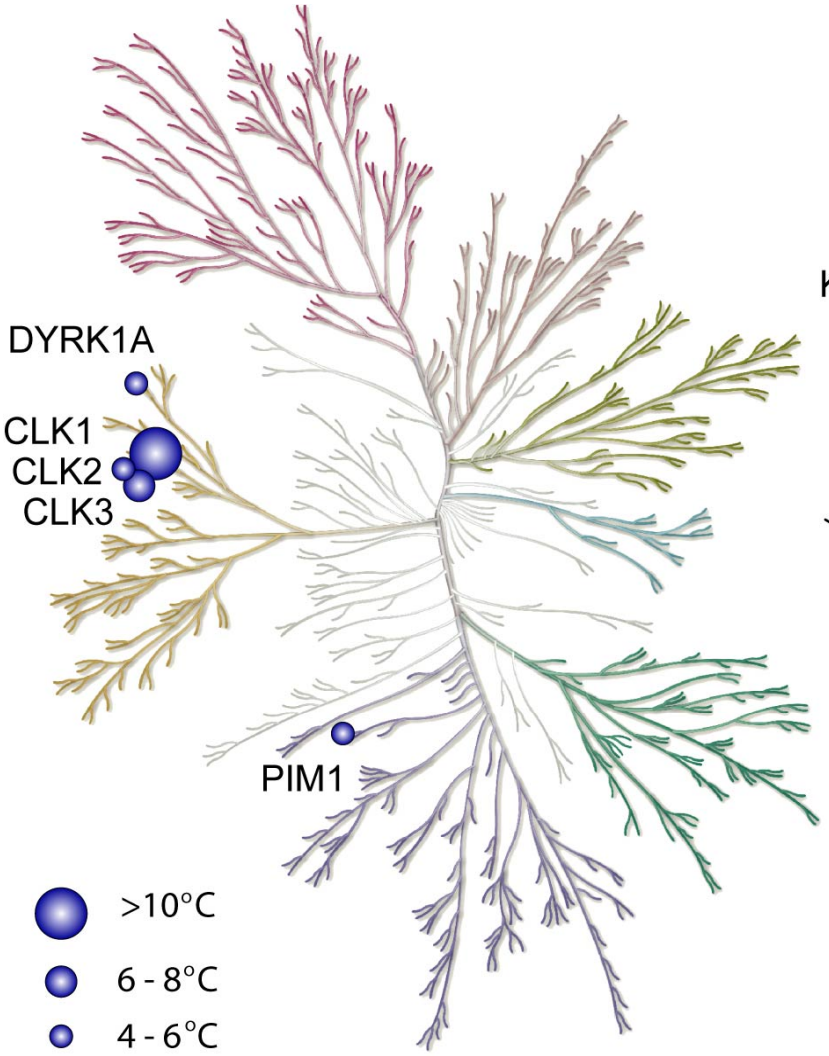


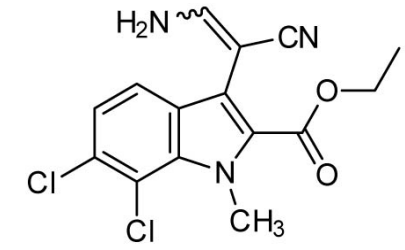
Selectivity can be increased by avoiding binding to ATP mimetic mode

Kinome wide analysis rationalizes most cross reactivity and suggests strategies for selective inhibitor development

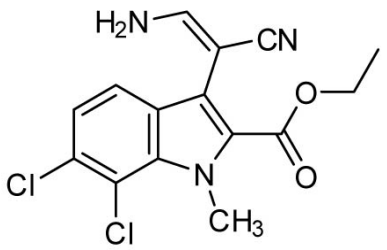


Targeting Splicing: CLK1

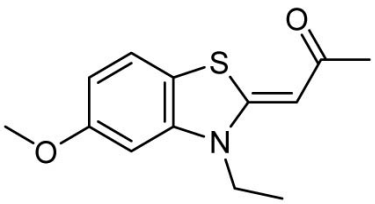




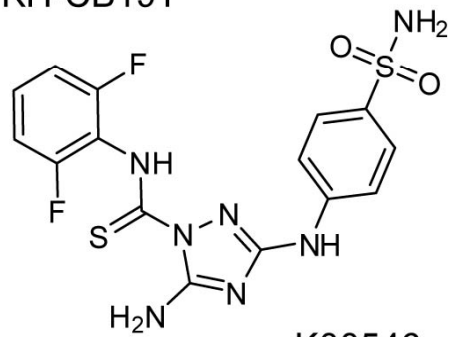
KH-CB20E



KH-CB19T



TG003



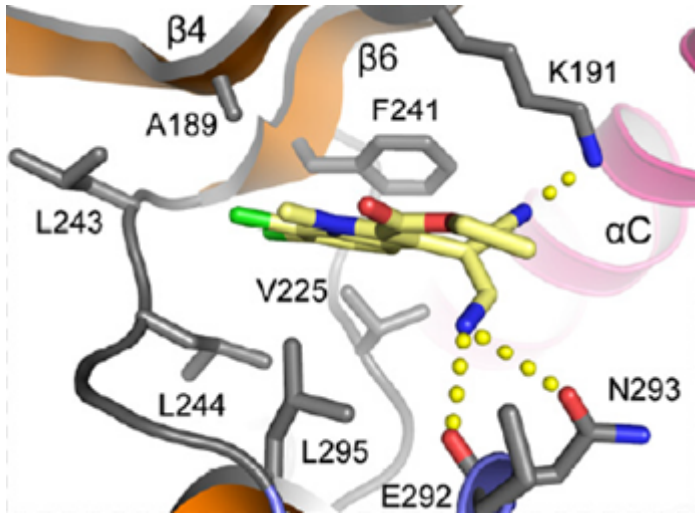
K00546

Inhibitor	CLK1 IC_{50}	CLK3 IC_{50}
KH-CB19T	19.7	1730
KH-CB20E	16.5	488
TG003	48.6	>4000
K00546	8.9	29.2

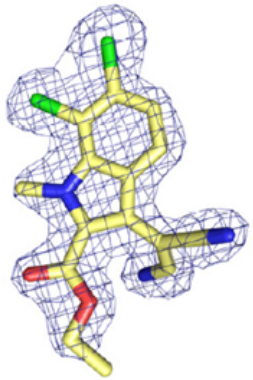
Selective for CLK1
(150 kinases screened by DSF
& 80 kinases screened by enzymatic assays)

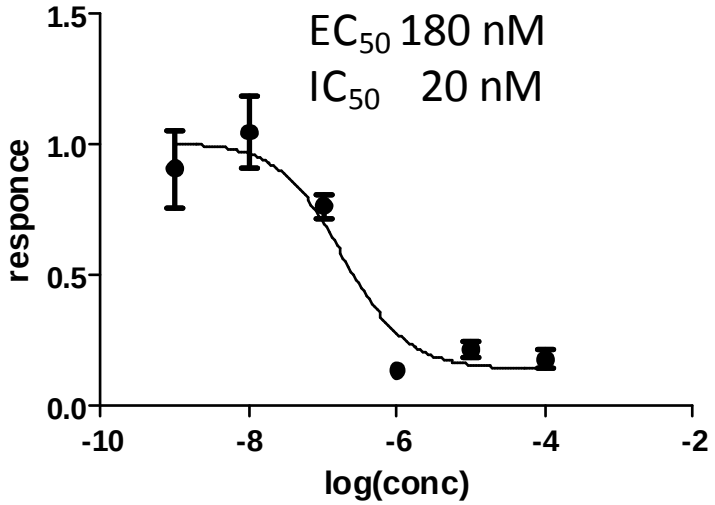
Collaboration : F. Bracher, University Munich

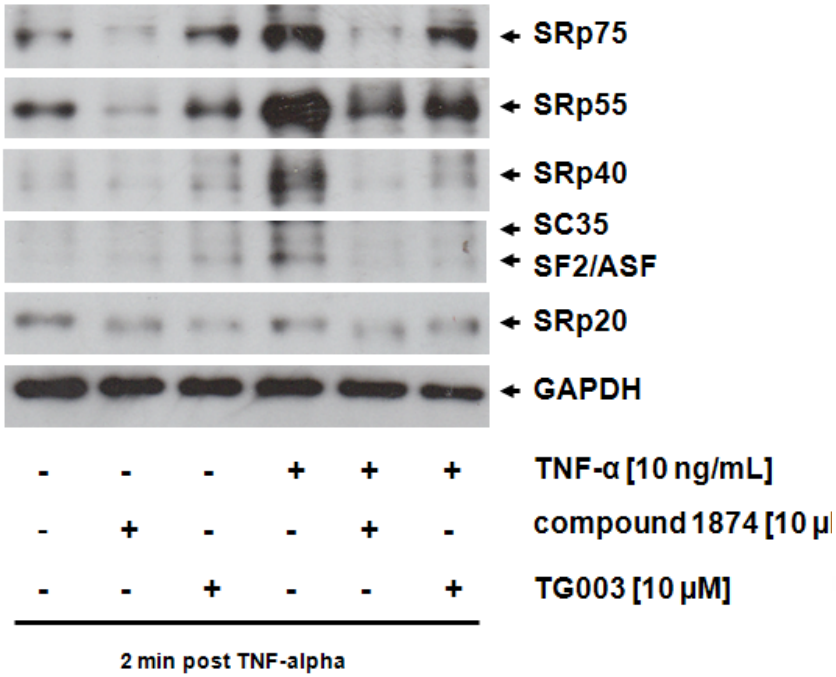
CLK1 Regulation of TF Splicing

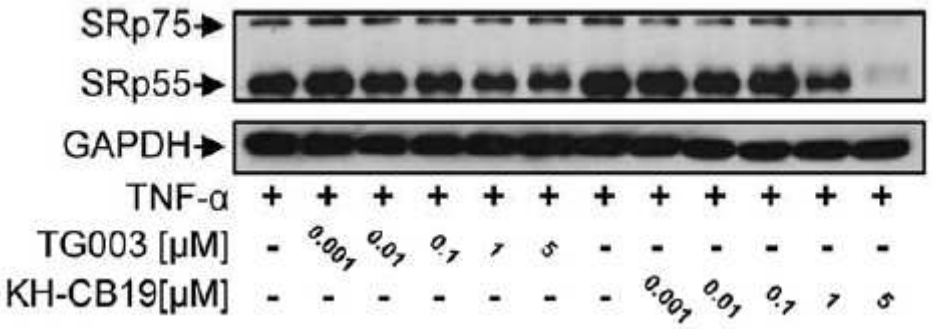


K01874 inhibits S/R phosphorylation in endothelia cells and splicing of TF.



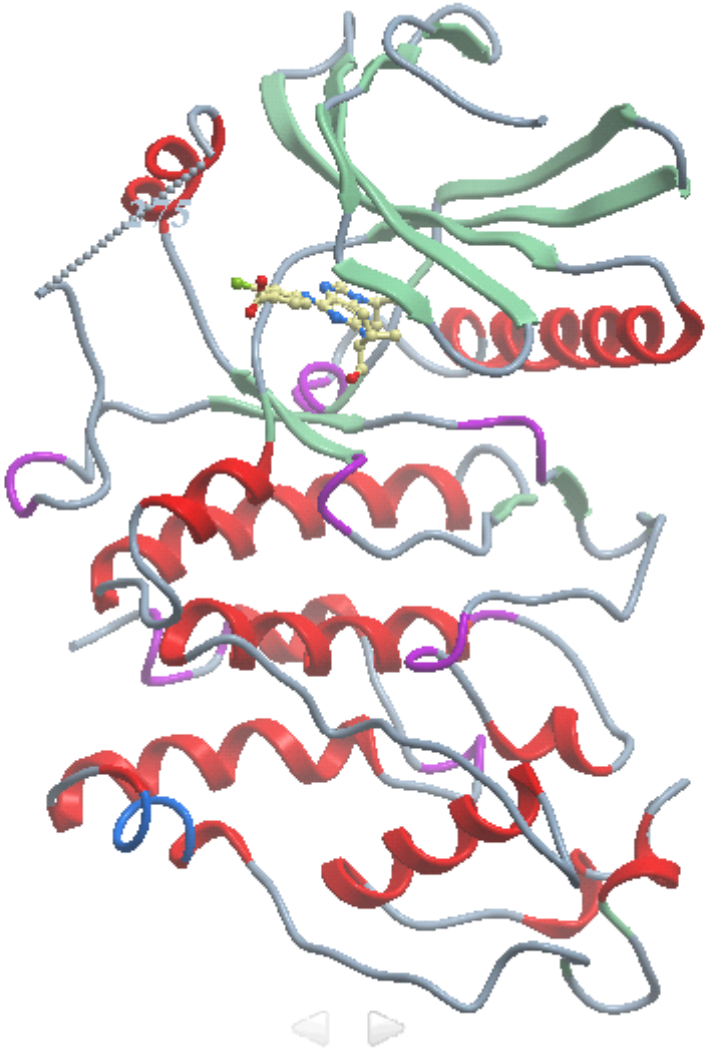




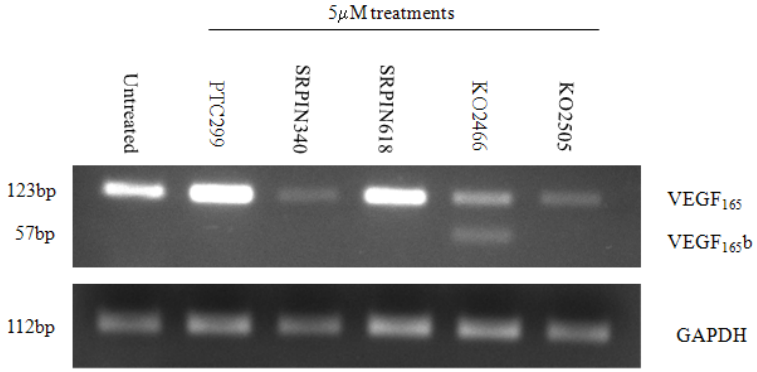


A. Eisenreich and U. Rauch Charite Berlin

SRPK2 Inhibitors and Regulation of VEGF Splicing



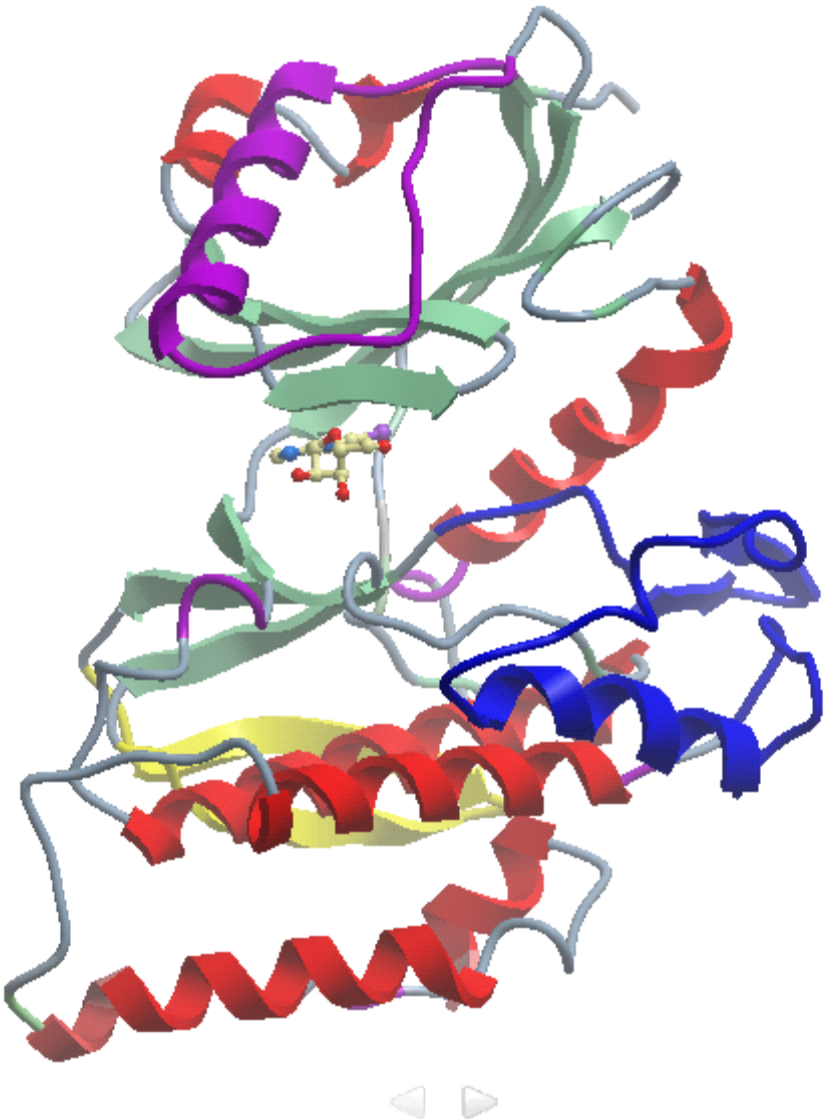
- **SRPK1/2** suppressed HCV replication
- **SPRK1** regulates vascular endothelial growth factor (VEGF) splicing from pro-angiogenic to anti-angiogenic isoforms

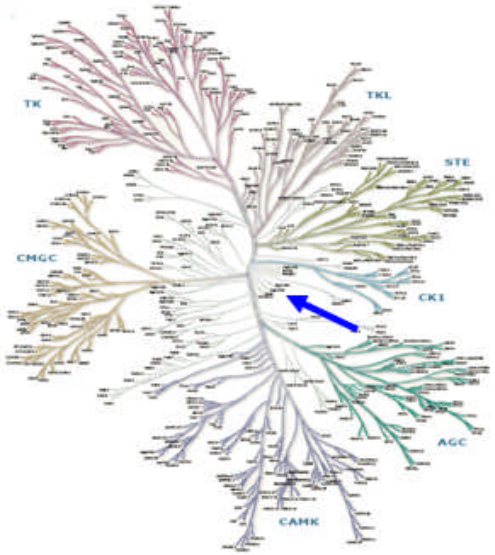


K02466 highly selective for SRPKs

Collaboration : D. Bates, Bristol ,UK

Targeting Unique Kinases (Haspin)

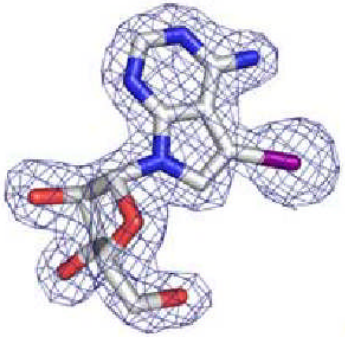




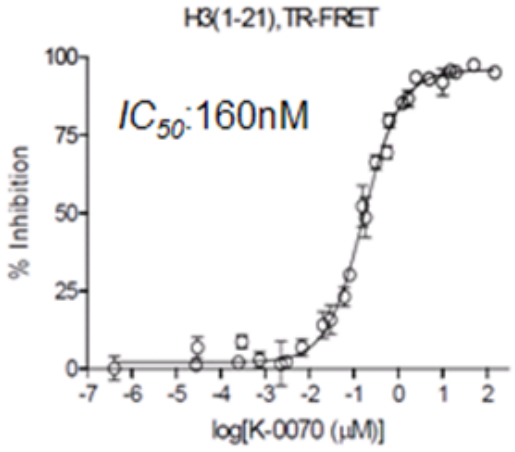
- **Low sequence homology** with ePKs (less than 20%)
- **Lack motifs** that are invariant in ePKs
- **Specific substrate**: H3T3
- **Depletion of Haspin** leads to:
  - Premature chromatid separation
  - Activation of spindle checkpoint
  - Block in mitosis
- **Activator of Aurora B**

Targeting Unique Kinases (Haspin)

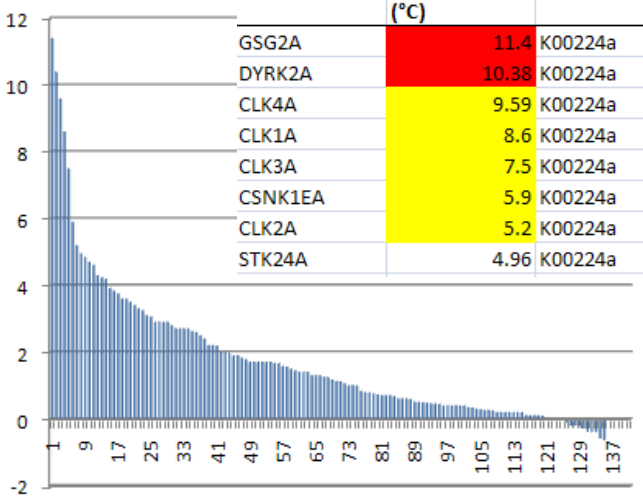
IC50: 5nM
in vitro

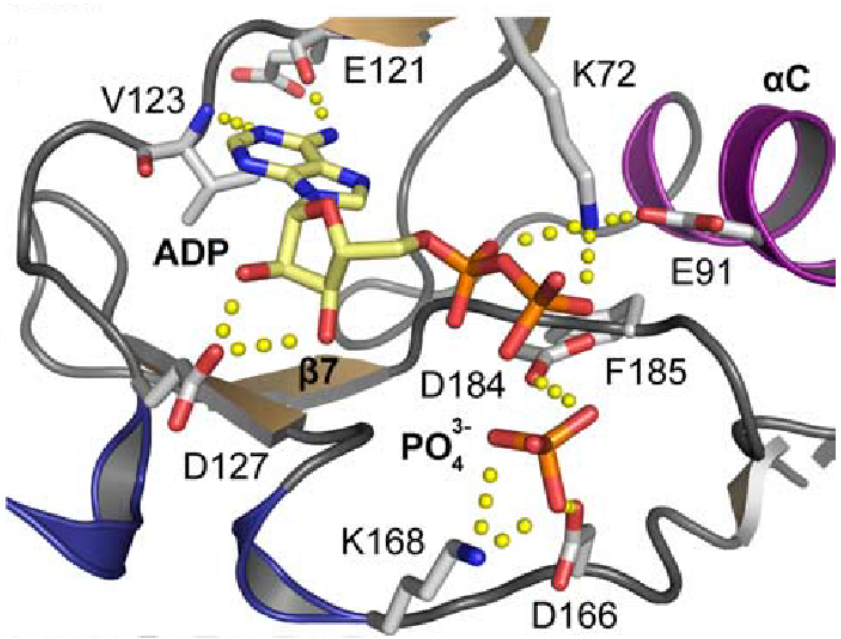


Iodotubercidin

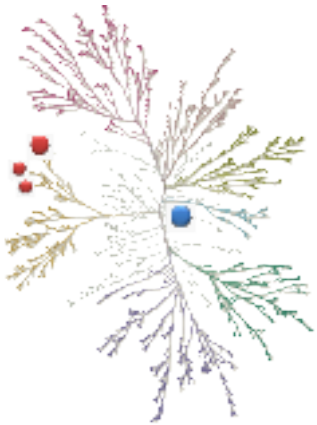


Target ID	CompScreen: Tm Shift (C) (°C)	Compound SGC ID	Protein Family
GSG2A	11.4	K00224a	Kinase-Other
DYRK2A	10.38	K00224a	Kinase-CMGC
CLK4A	9.59	K00224a	Kinase-CMGC
CLK1A	8.6	K00224a	Kinase-CMGC
CLK3A	7.5	K00224a	Kinase-CMGC
CSNK1EA	5.9	K00224a	Kinase-CK1
CLK2A	5.2	K00224a	Kinase-CMGC
STK24A	4.96	K00224a	Kinase-STE

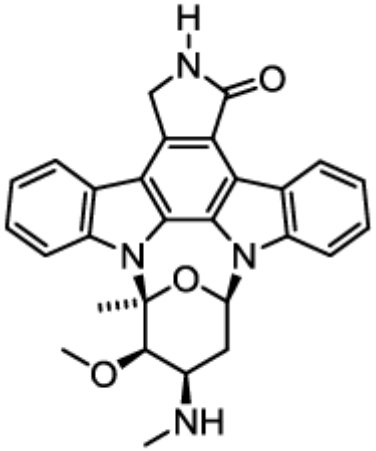




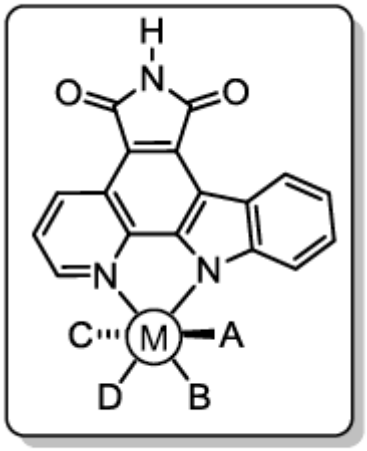
- 137 kinases screened
- Only cross reactivity that has been observed is to DYRK2 and CLK kinases



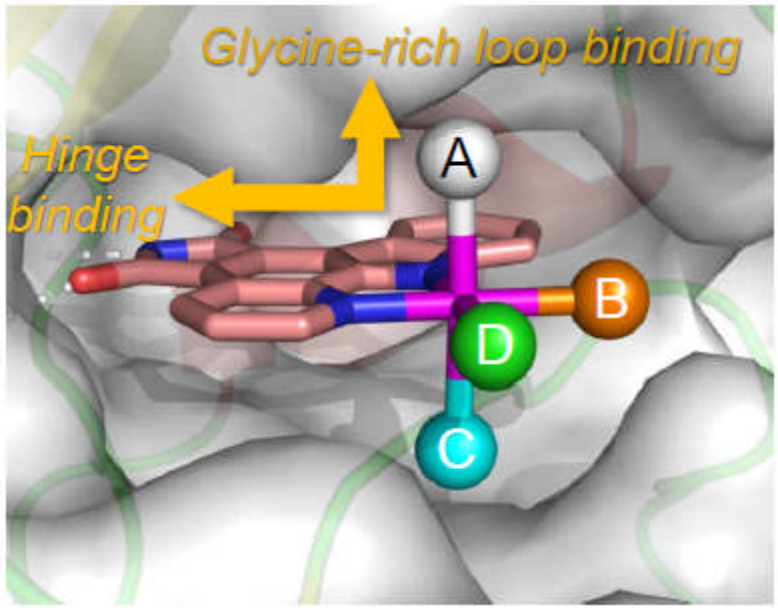
New Approaches: Octasporines

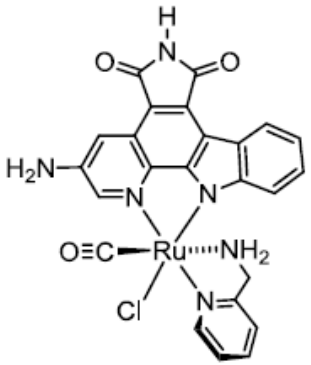


Staurosporine

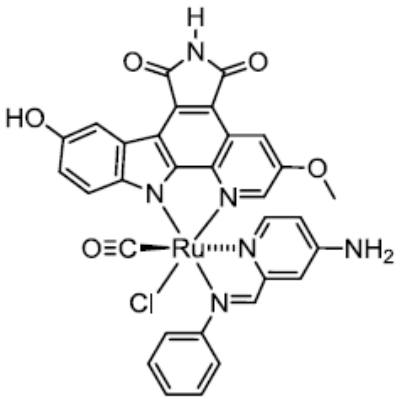


Octasporines (**OS**)

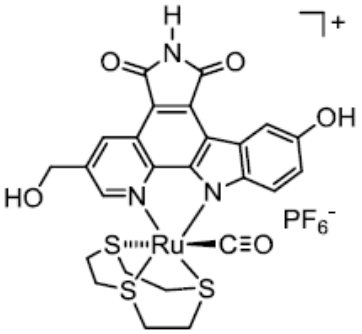




Λ-**OS1**, GSK3α inhibitor
0.9 nM



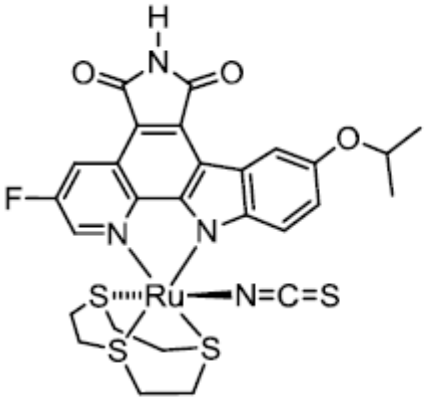
Λ-**OS2**, PAK1 inhibitor
350 nM



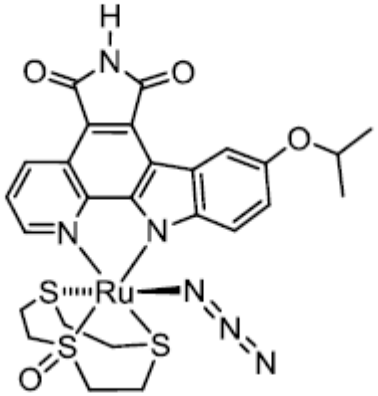
OS3, Pim1 inhibitor
0.08 nM

Collaboration : E. Meggers (Marburg)

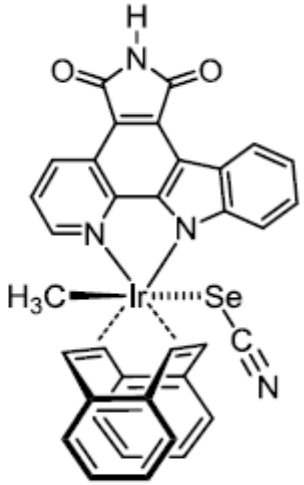
New Approaches: Octasporines



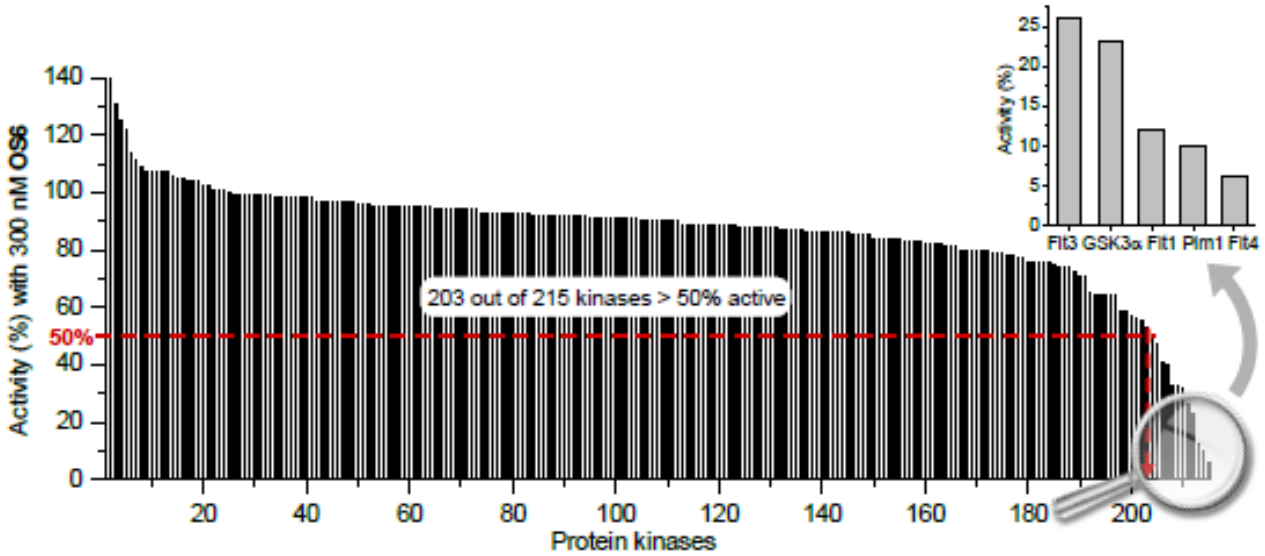
OS4, DAPK1 inhibitor
2.0 nM



OS5, MLCK inhibitor
4.4 nM



OS6, FLT4 inhibitor
42 nM



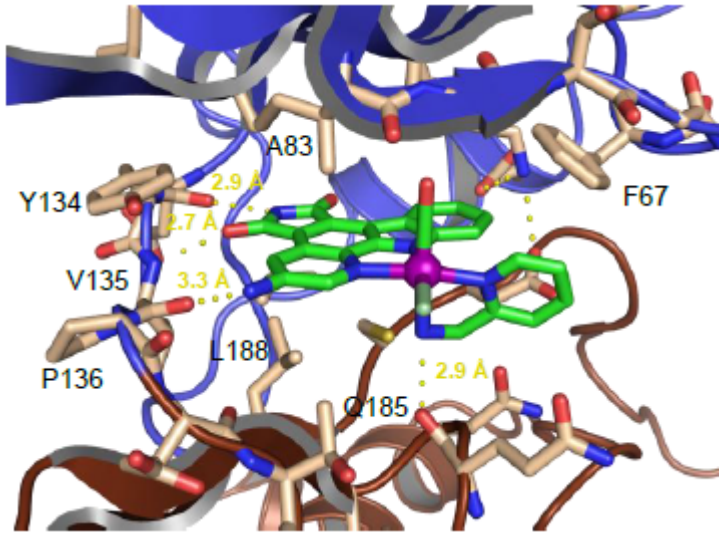
Collaboration : E. Meggers (Marburg)

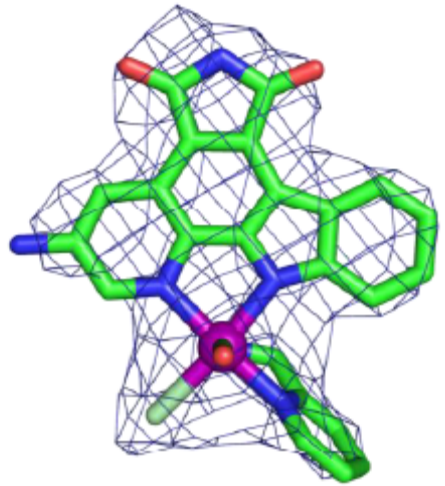
New Approaches: Octasporines

Table 1. IC_{50} data (nM) for octahedral kinase inhibitors at 100 µM ATP[a]

	GSK3α	PAK1	Pim1	DAPK1	MLCK	FLT4
Λ-OS1	0.9	>100000	14	22800	22	1180
Λ-OS2	2000	350	1570	>30000	24300	2300
OS3	20	82	0.075	315	2.2	29
OS4	>100000	>100000	169	2.0	25	163
OS5	31000	>100000	435	113	4.4	48
OS6	3900	10000	333	>100000	>30000	42

[a]IC_{50} data were obtained by phosphorylation of substrates with [γ-^{33}P]ATP and 100 µM ATP in the presence of different concentrations of octasporine kinase inhibitors. IC_{50} values represent the average of three independent measurements. Error bars are within 20 % of the determined values.





➢ Octasporines are highly potent & target selective

Collaboration : E. Meggers (Marburg)

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