Covalent and Non-Covalent Lead Generation Strategies for VRK1, a Paralog Synthetic Lethal Target in Cancer

Kasia Handing, Ph.D.

2026.09.29

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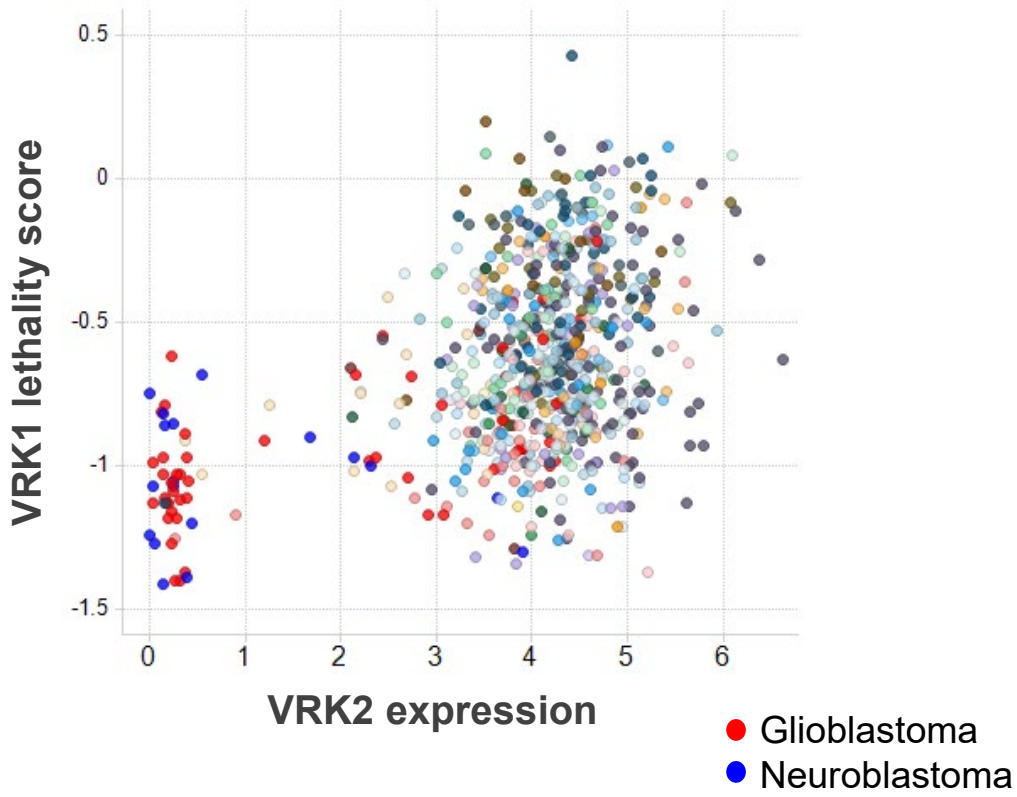
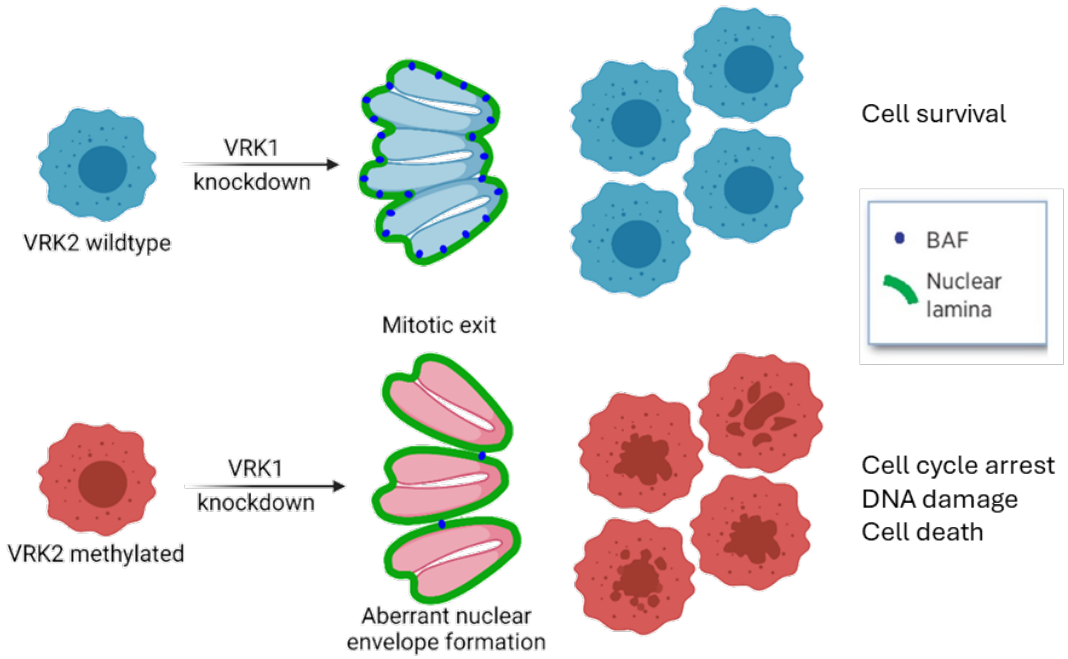
VRK1 is a synthetic lethal kinase target in VRK2-low brain cancer

Approximately 66% of malignant brain tumors have low VRK2 expression



VRK1 knockdown leads to cell cycle arrest, accumulation of DNA damage, and cell death



Shields, J. A. *et al. Cancer Res.* **82**, 4044- 4057 (2022).

In **VRK2-low cancers** (such as promoter-methylated glioblastomas and neuroblastomas), cell survival becomes entirely dependent on the sister kinase **VRK1**.

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VRK1 is a synthetic lethal kinase target in VRK2-low brain cancer

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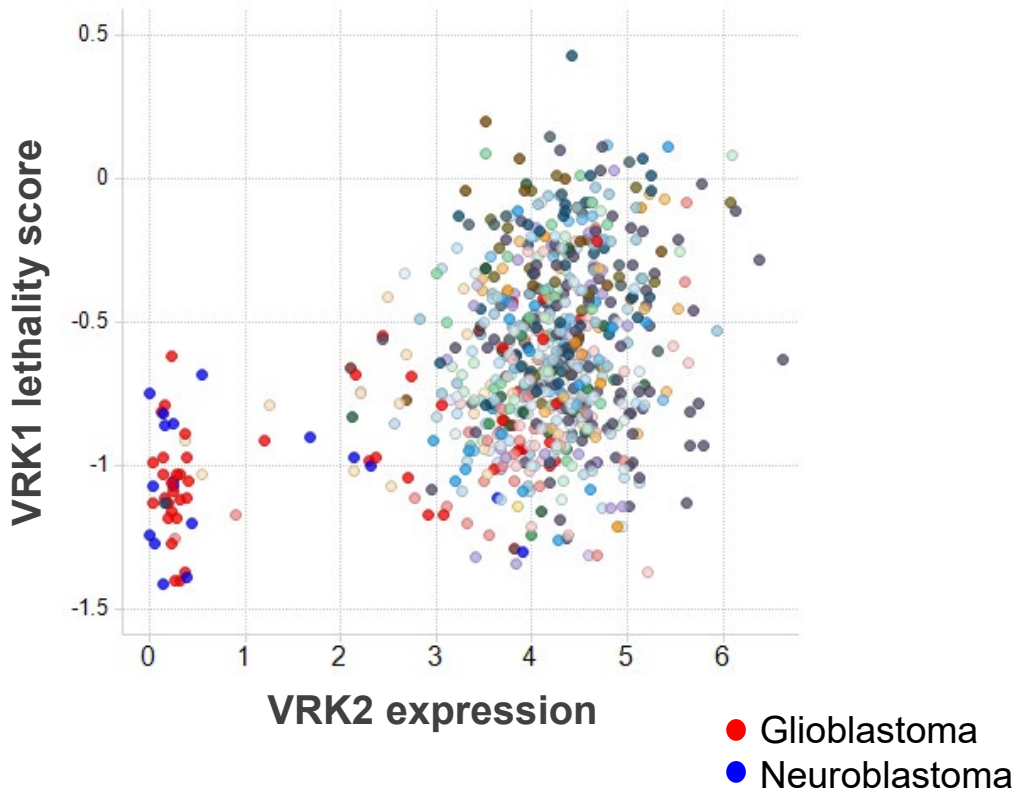
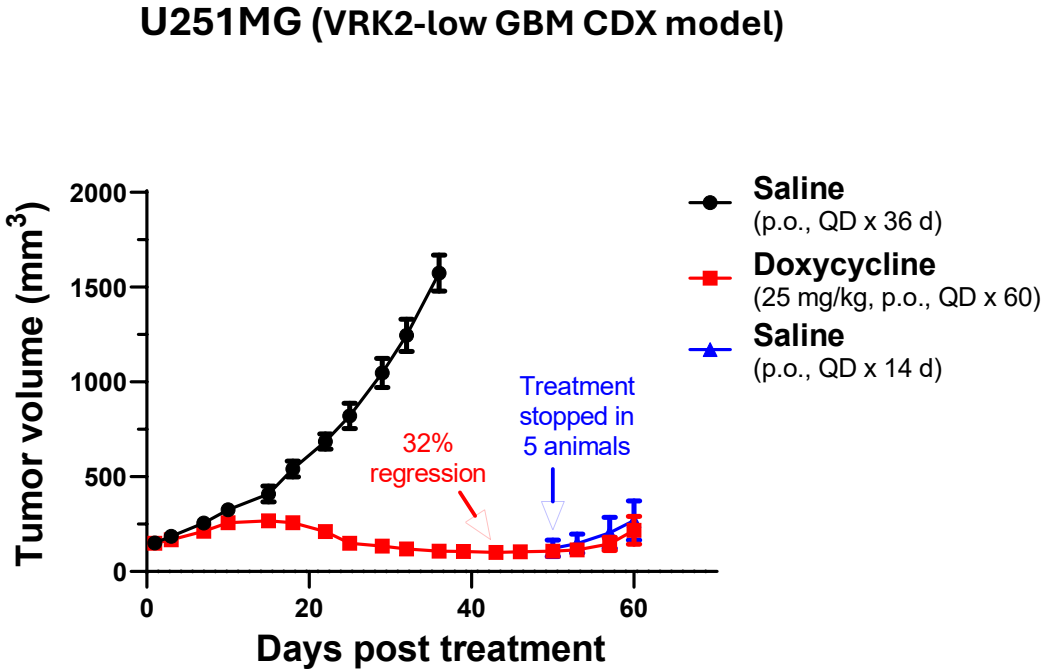


Dox-inducible CRISPRi-based VRK1 knockdown drives tumor regressions in VRK2-low tumors



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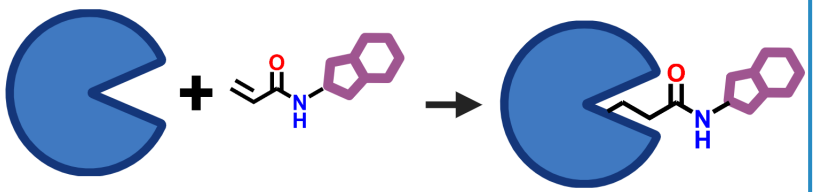
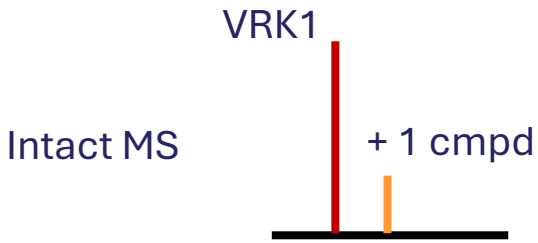
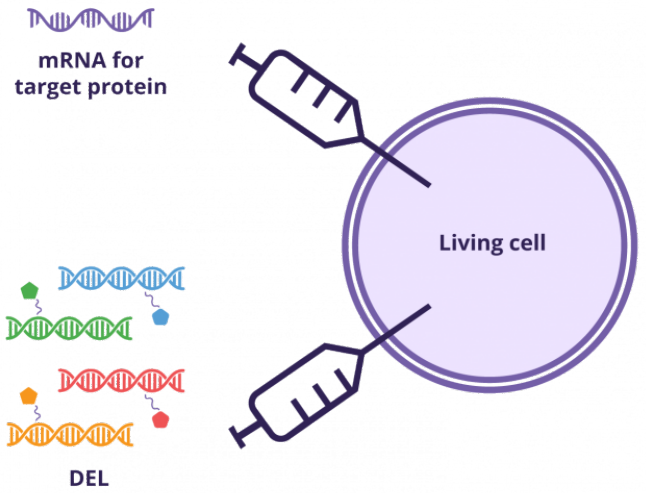
Multiple hit-finding approaches explored for VRK1

Covalent biochemical screen



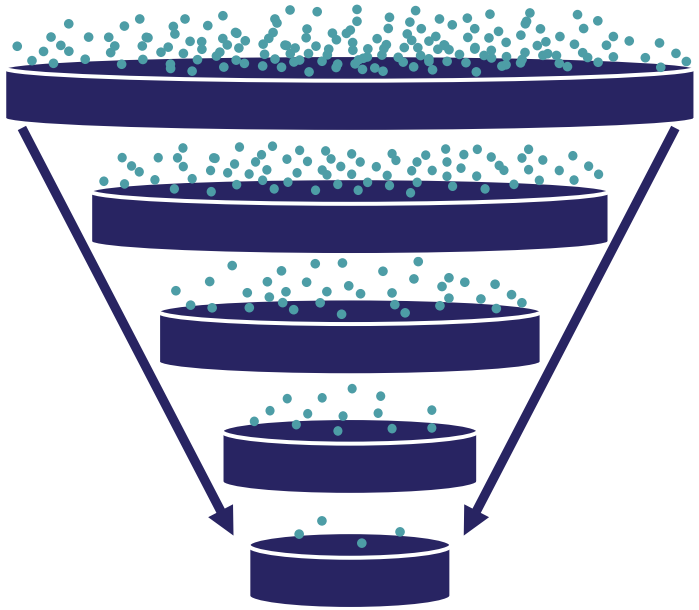


Cellular Vipergen DEL screen



https://www.vipergen.com/

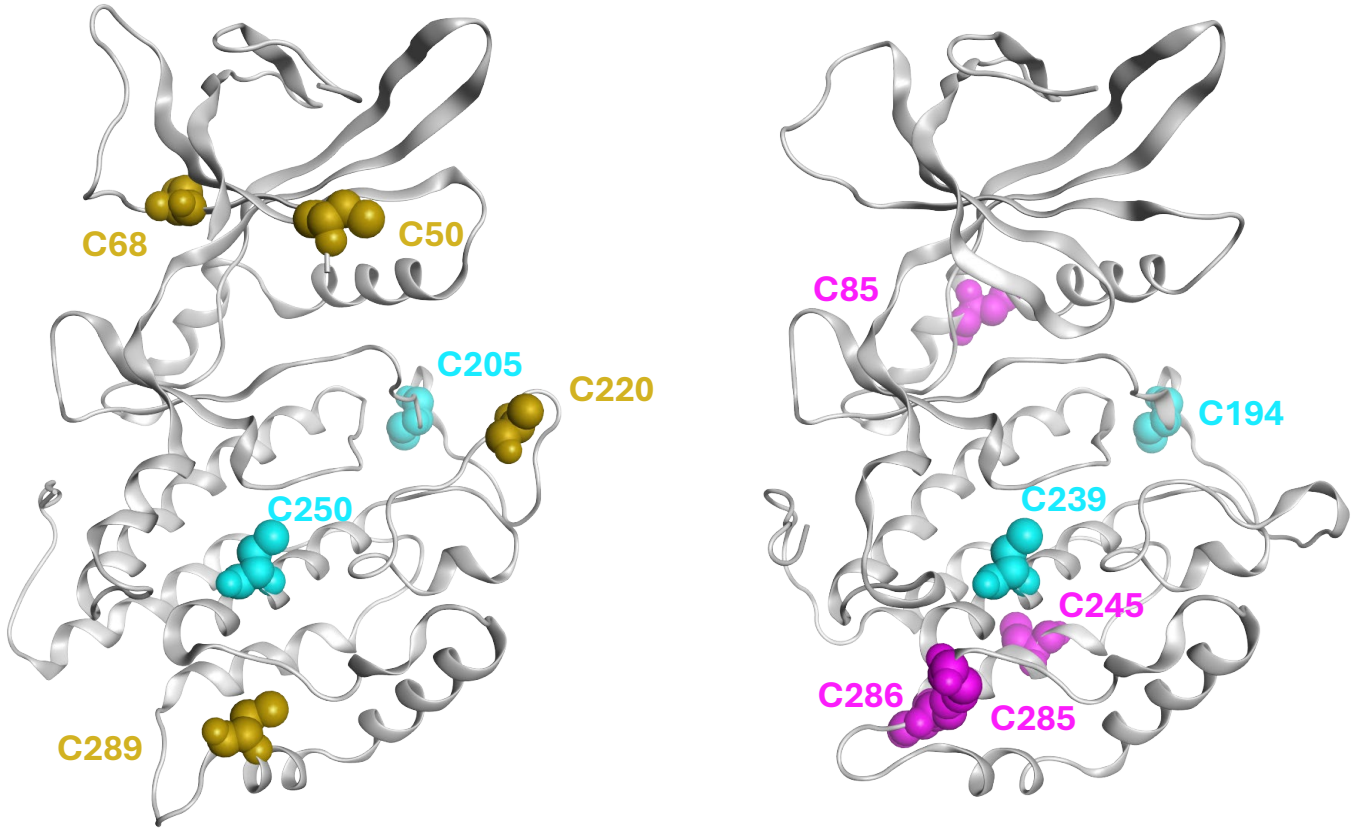
Other approaches



Exploiting unique cysteine residues for covalent, paralog-selective VRK1 inhibition

VRK1	VRK2	Covalent screen

57% sequence identity across catalytic domains



Cys residues unique to VRK1

Cys residues unique to VRK2

Cys residues conserved between VRK1 and VRK2

Library of ~6000 acrylamides

Average MW = 350
90% ≤ 2 HBDs

TR-FRET biochemical assay
117 compounds with IC_{50} < 200 µM

MS assay
6 compounds with 70% single adduct formation in VRK1

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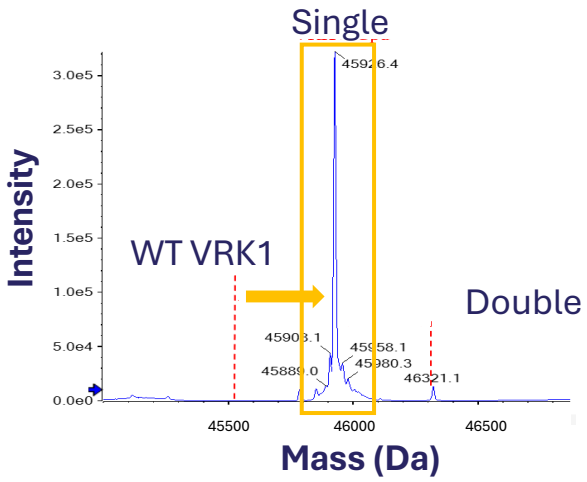
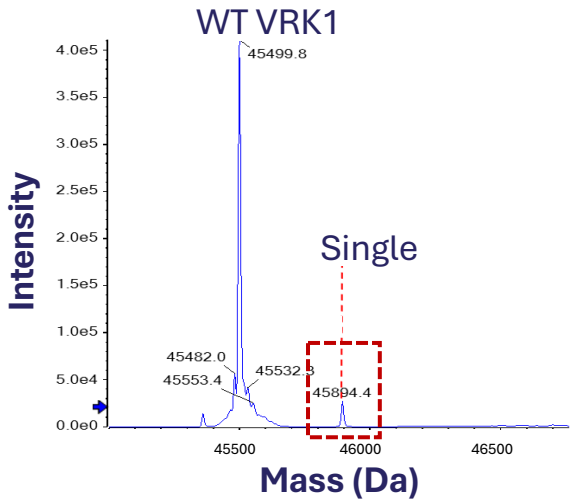
Mass spectrometry and mutational analysis confirms Cys50 as the primary covalent labeling site for VRK1

WT VRK1



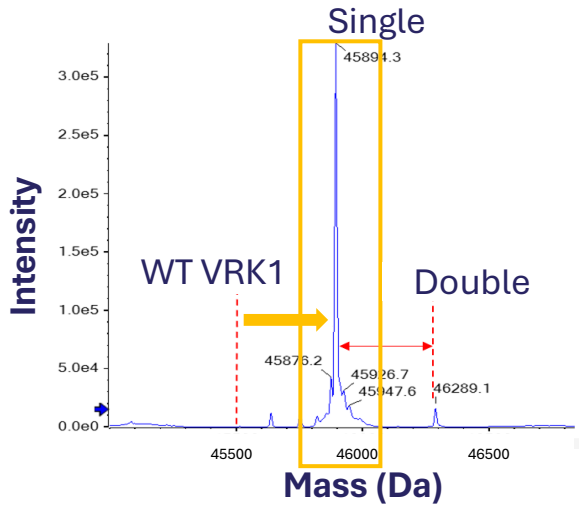
Labeling efficiency (%)	
Single	96.1
Double	3.9

C50A VRK1



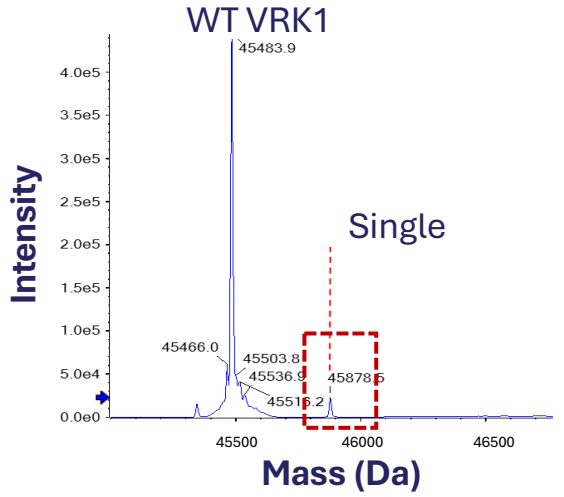
Labeling efficiency (%)	
Single	6.2

C68A VRK1



Labeling efficiency (%)	
Single	95.3
Double	4.7

C50A/C68S VRK1



Labeling efficiency (%)	
Single	4.9

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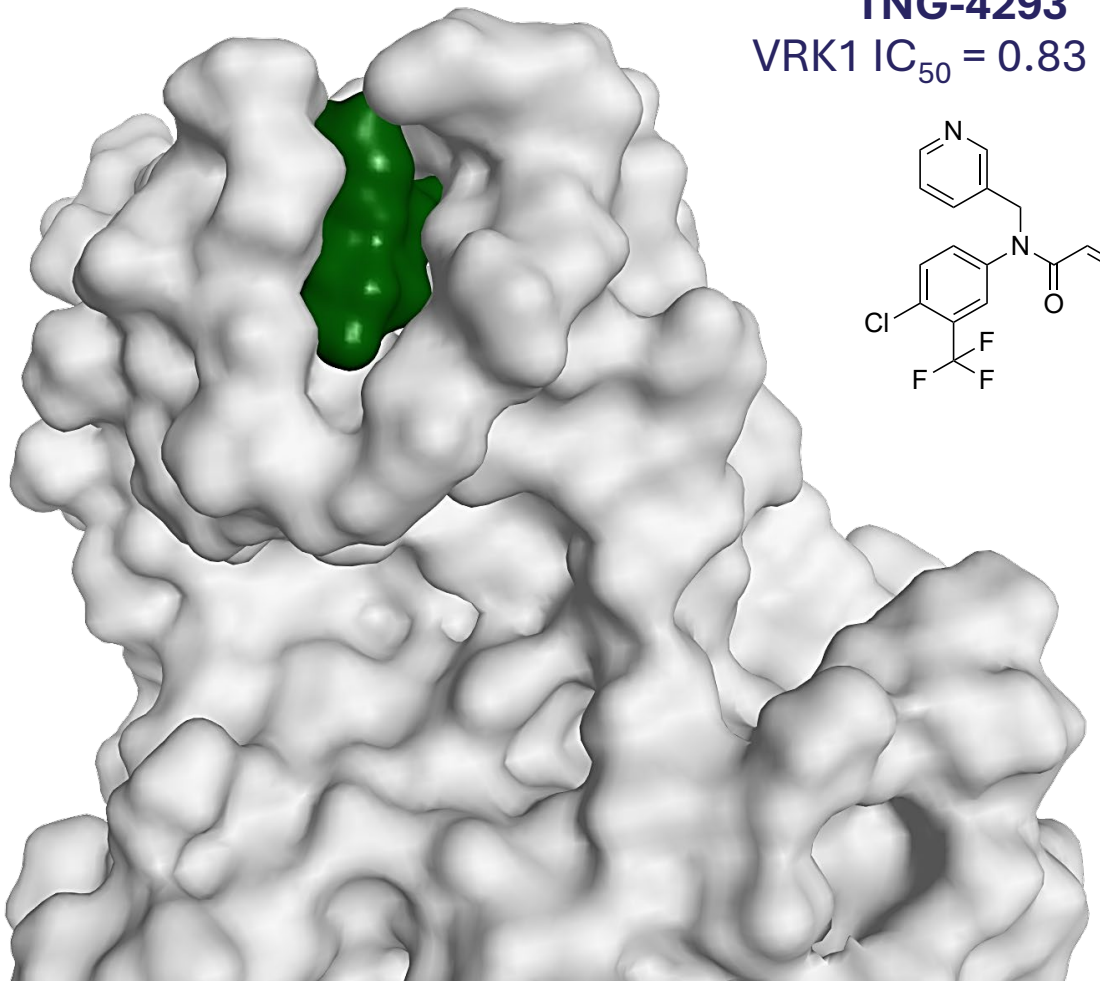
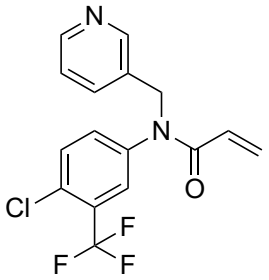
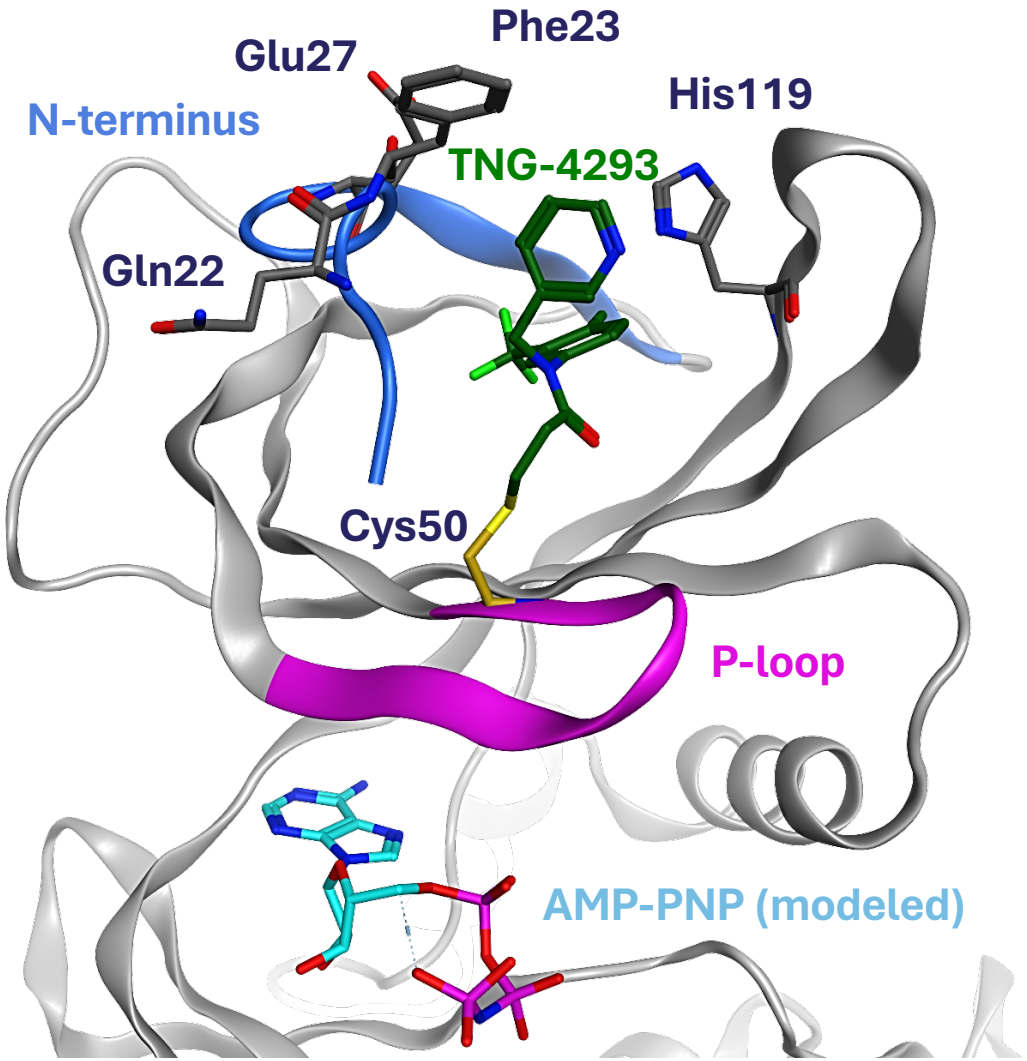
Structural studies of TNG-4293 confirm Cys50 engagement and reveal a novel VRK1 site



TNG-4293

VRK1 IC_{50} = 0.83 µM



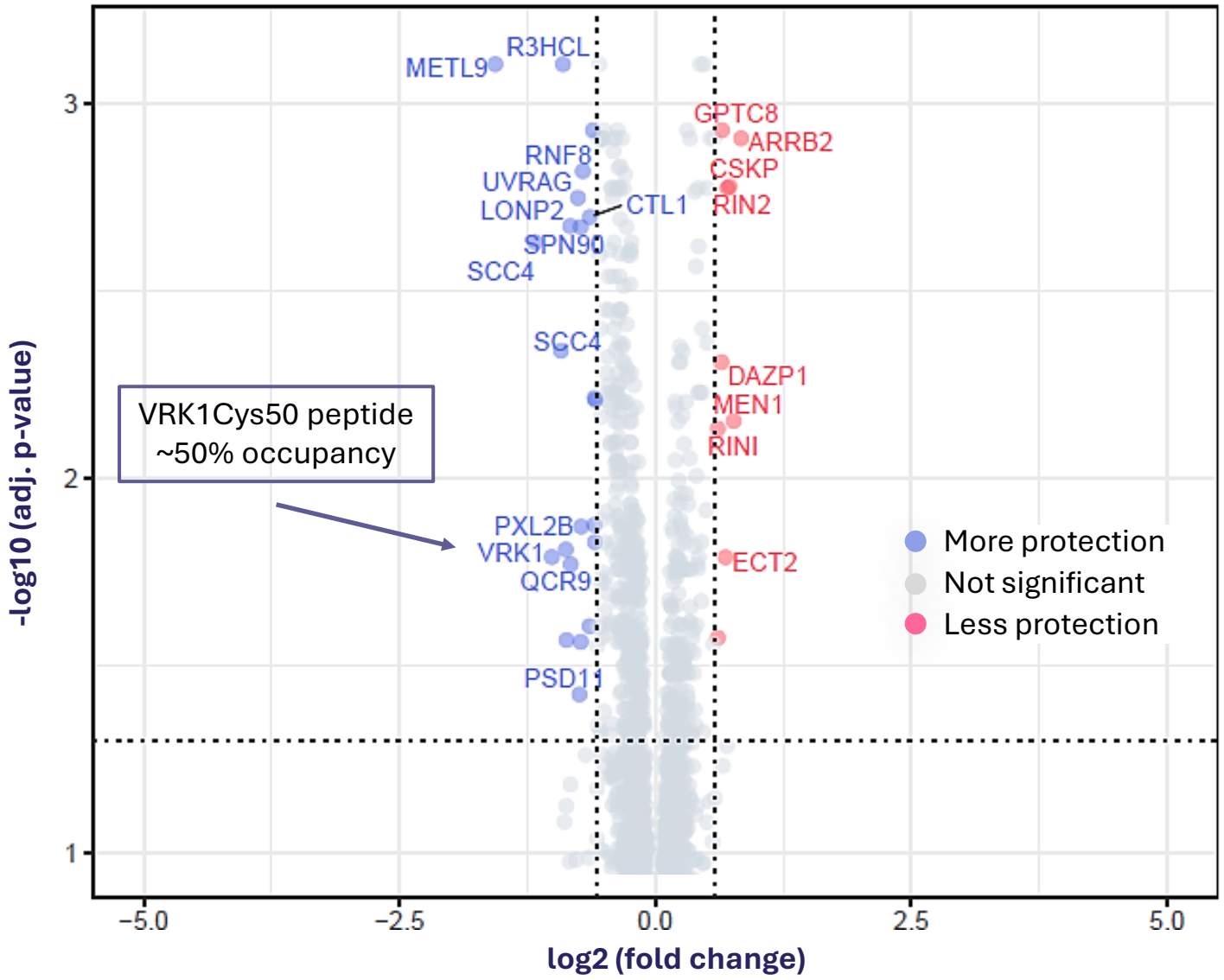


Resolution = 2.1 Å

R_{work} / R_{free} = 0.18 / 0.23

TANGO therapeutics

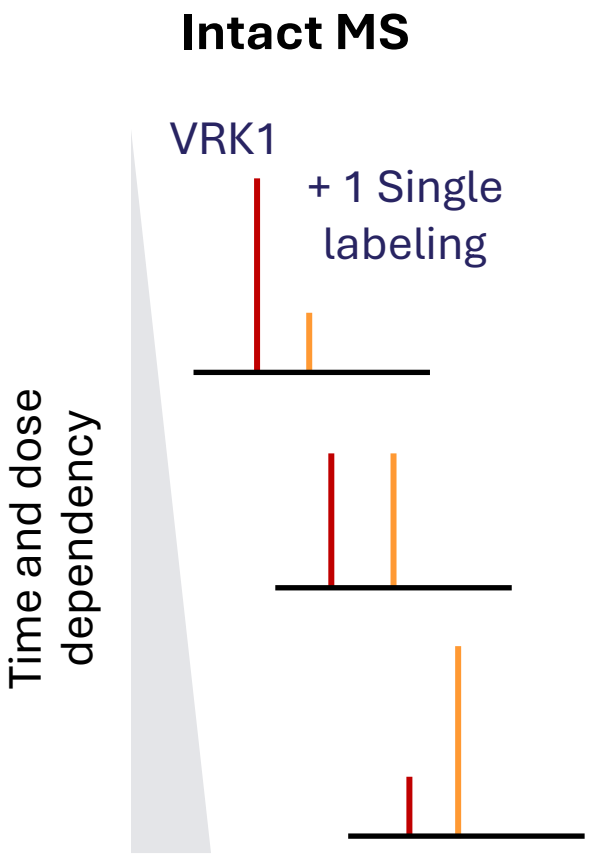
TNG-4293 labels VRK1 in cells but exhibits broad proteome-wide reactivity

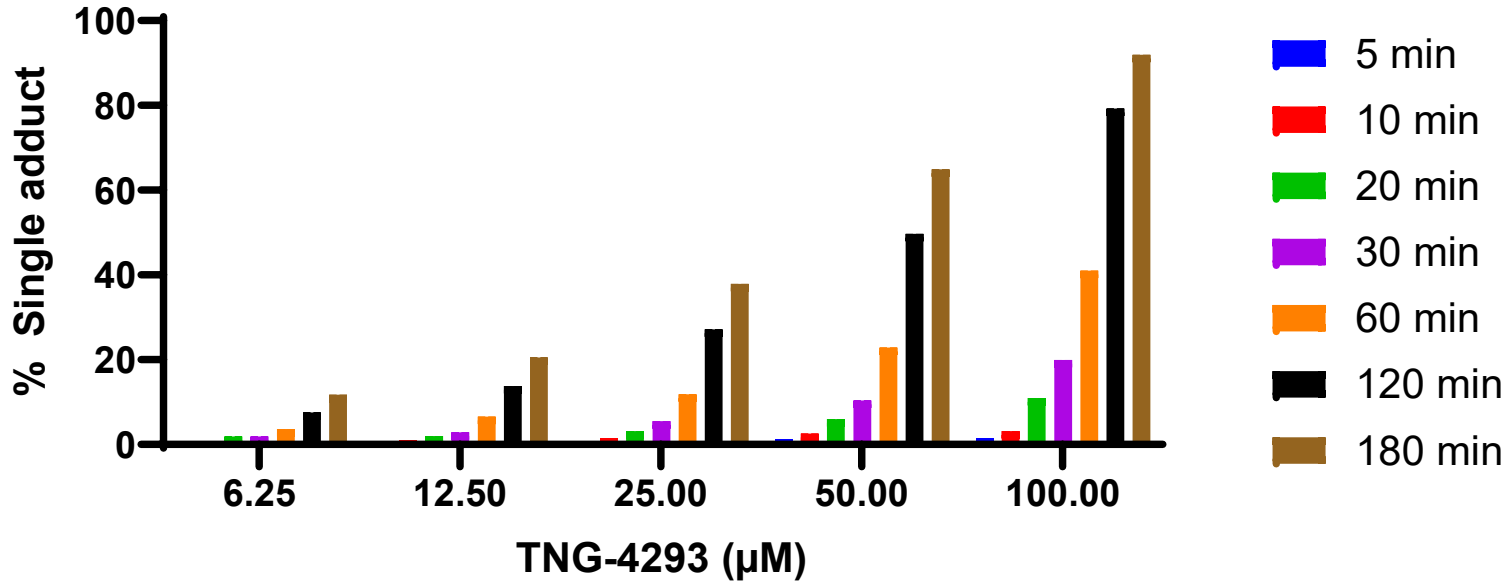


Compound: TNG-4293
Cells: U251MG
Digestion Enzyme: Trypsin + Asp-N

Potency is driven by warhead reactivity rather than binding affinity

Time and dose dependent adduct formation observed





%Adduct Formation
=
VRK1+ 1 single labeling / total VRK1

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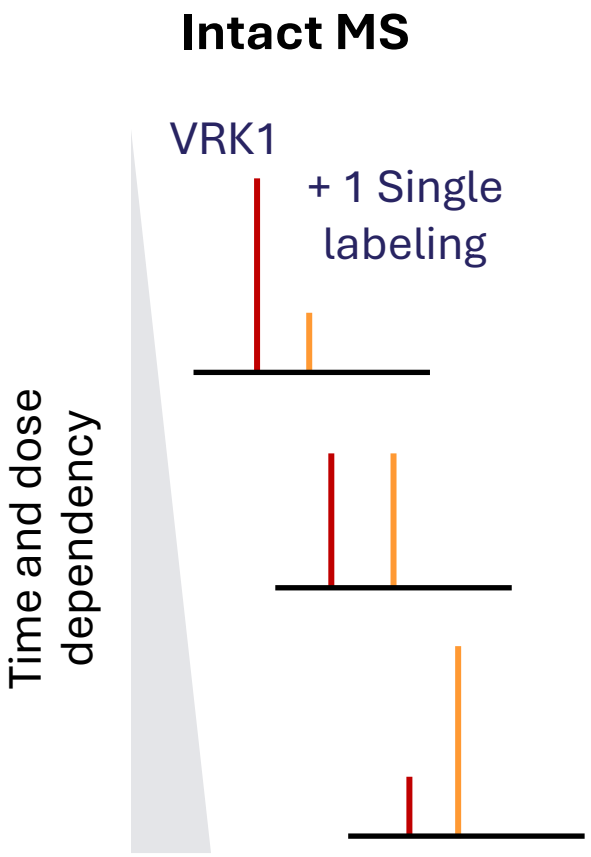
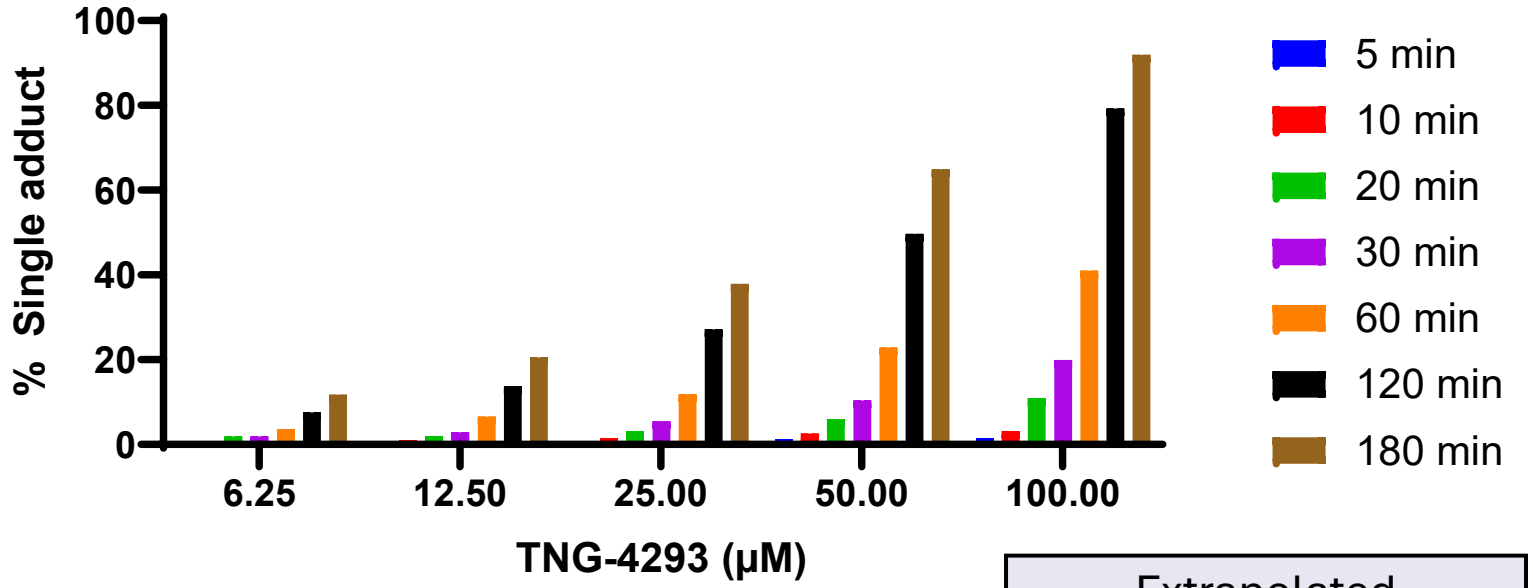
Time and dose dependent adduct formation observed

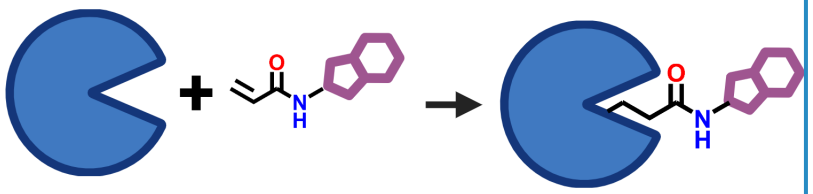


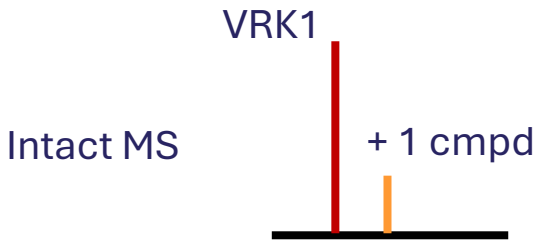


ID	IC_{50} (μM)	k_{inact}/K_i ($M^{-1}s^{-1}$)	Extrapolated	
			k_{inact} (s^{-1})	K_i (mM)
TNG-5008	41.2	1.31	0.183	139
TNG-4293	0.83	94.96	13.2	139

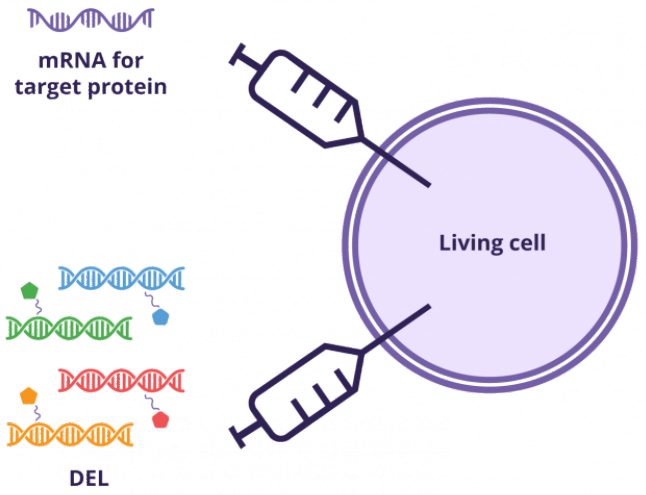
Multiple hit-finding approaches explored for VRK1

Covalent biochemical screen





Cellular Vipergen DEL screen



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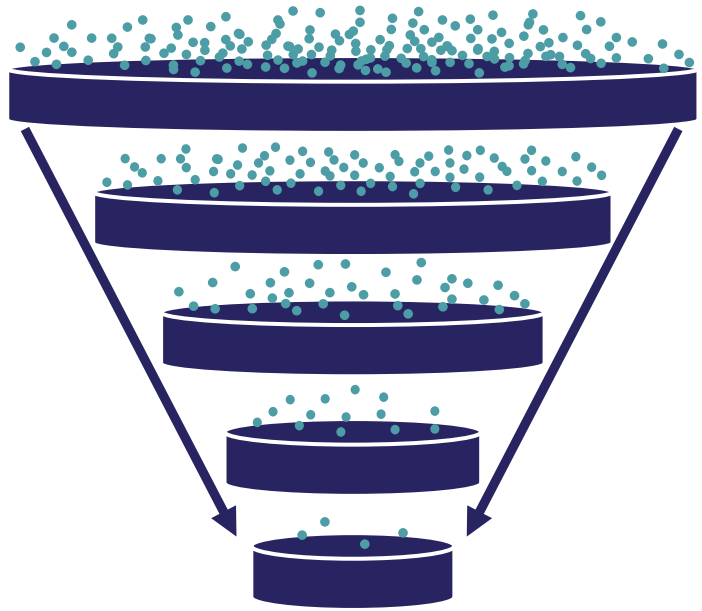
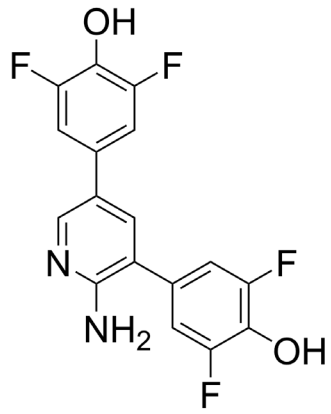
Other approaches



Limited chemical diversity of known VRK1 inhibitors



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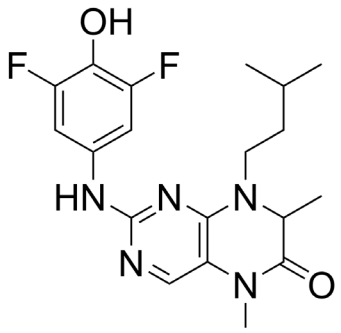
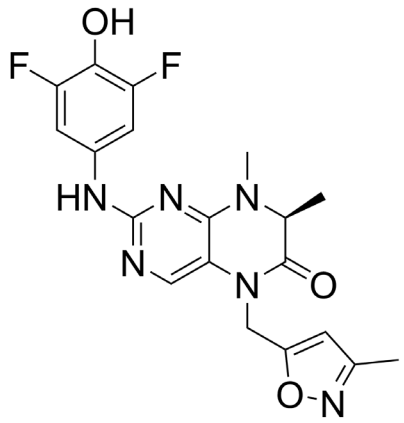
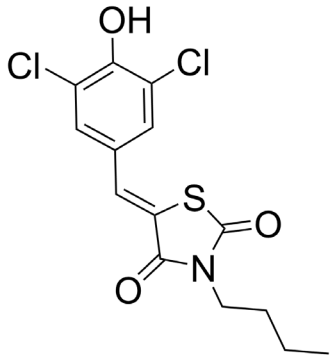


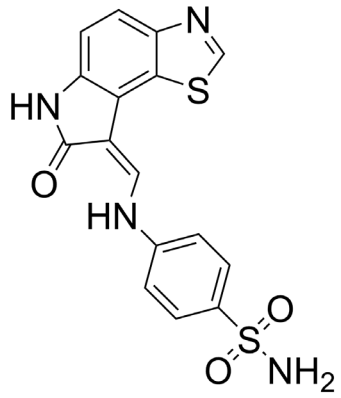
5UVF



8V42



9ZKG



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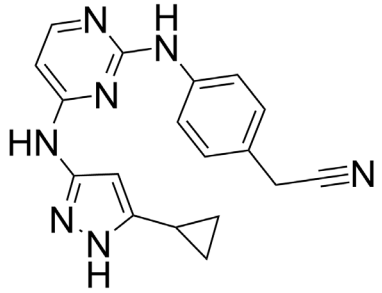
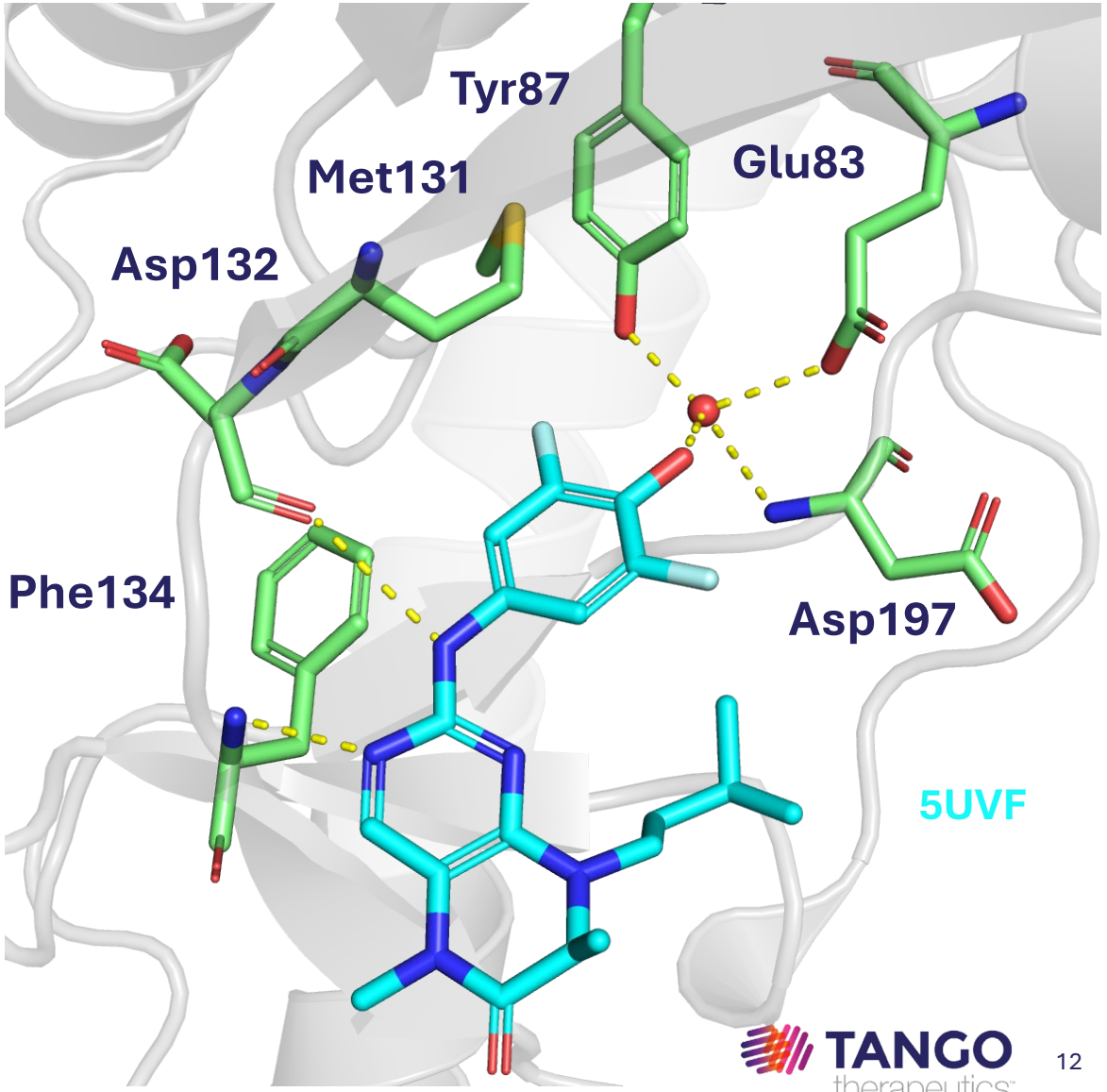


3OP5



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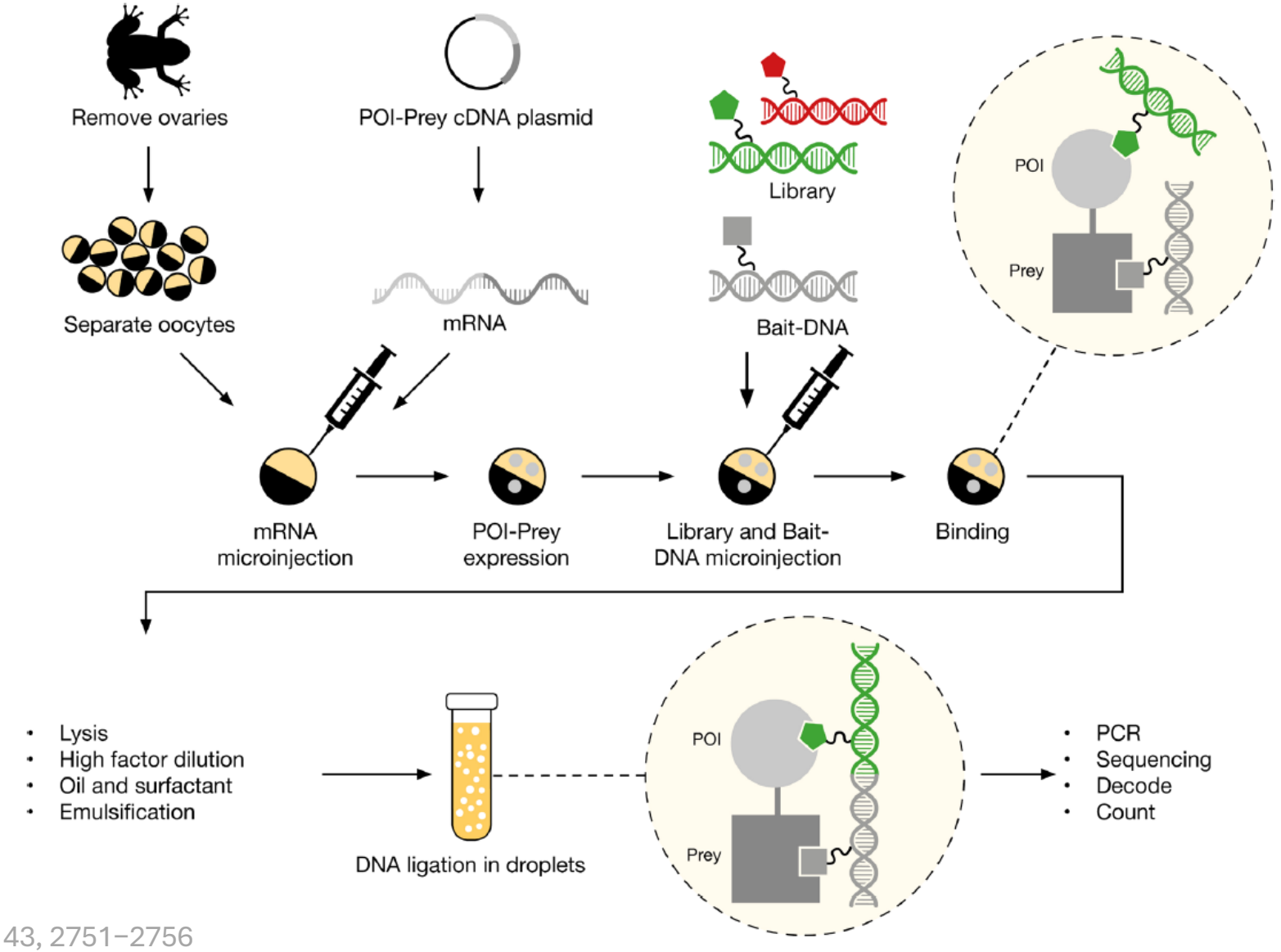
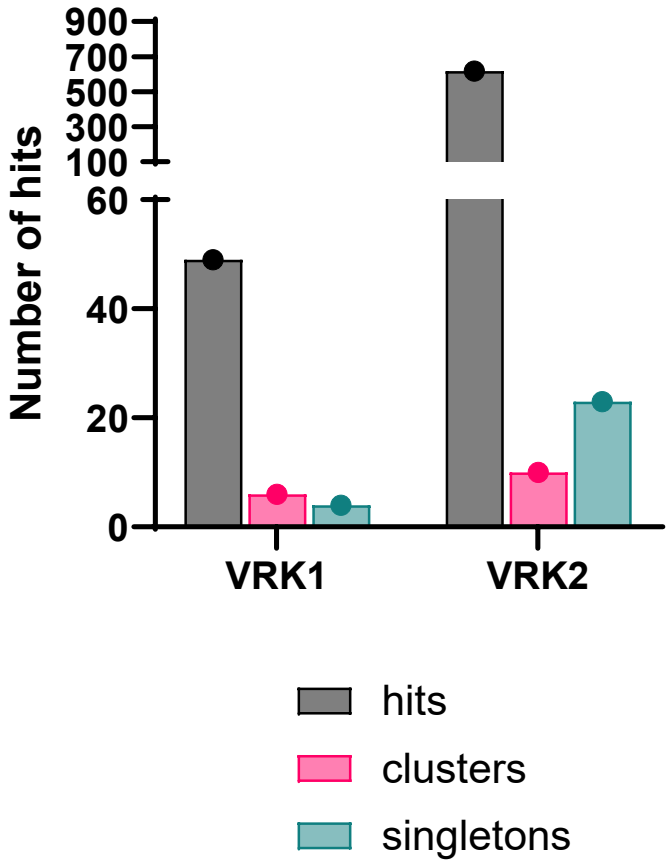
Principle of cellular Vipergen DEL screening

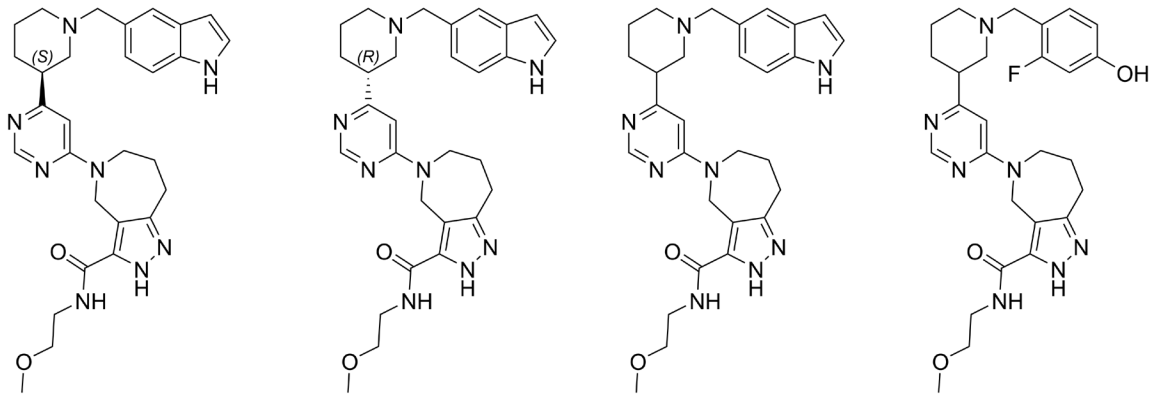


J. Am. Chem. Soc. 2021, 143, 2751−2756

Selective and cell active compounds identified from DEL screen

Hits identified for VRK1 and VRK2

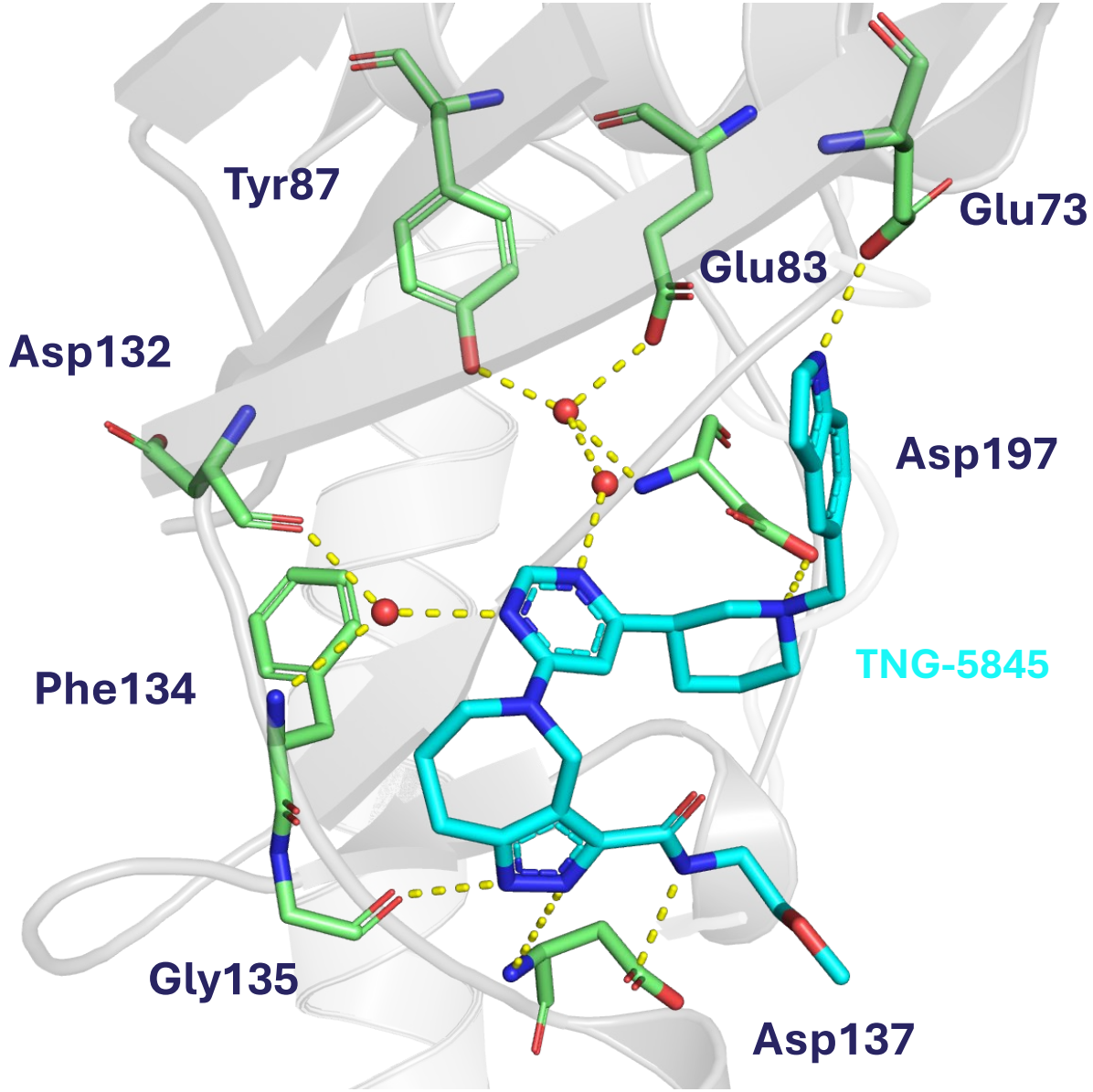


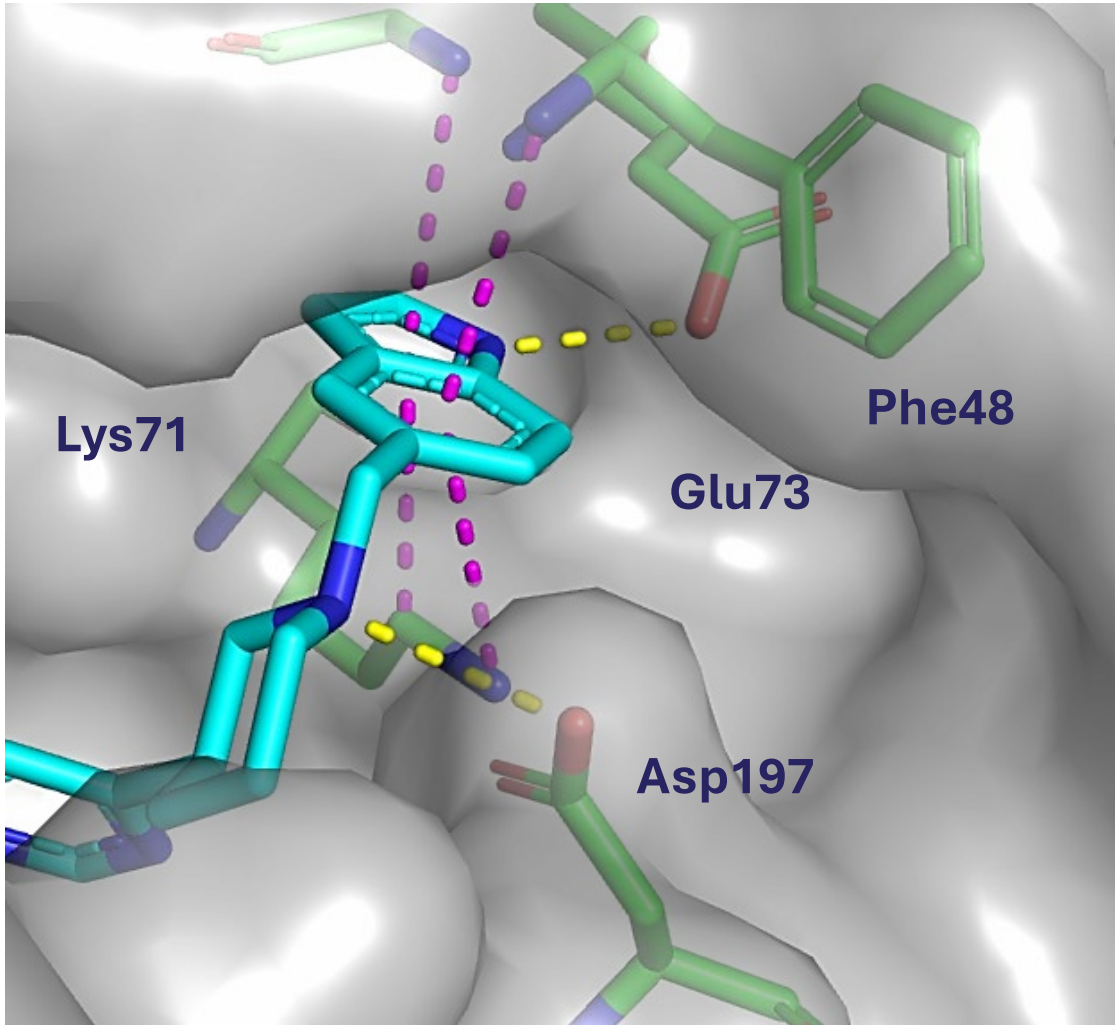


	TNG-5845	TNG-5844	TNG-6992	TNG-5296
VRK1 IC_{50} (μM)	0.079	1.96	0.033	4.43
VRK2 IC_{50} (μM)	1.6	10.7	-	> 30
nanoBRET IC_{50} (μM)	0.895	2.98	-	13.4

IC_{50} values reported with VRK1 TR-FRET assay with ATP concentration equal to K_m for VRK1 (4.5 μM). VRK1 cellular IC_{50} reported from nanoBRET assay in U251MG VRK2-low GBM cell line.

Crystal structure of TNG-5845 reveals water-mediated hinge interaction





Resolution = 2.4 Å

R_{work} / R_{free} = 0.18 / 0.24

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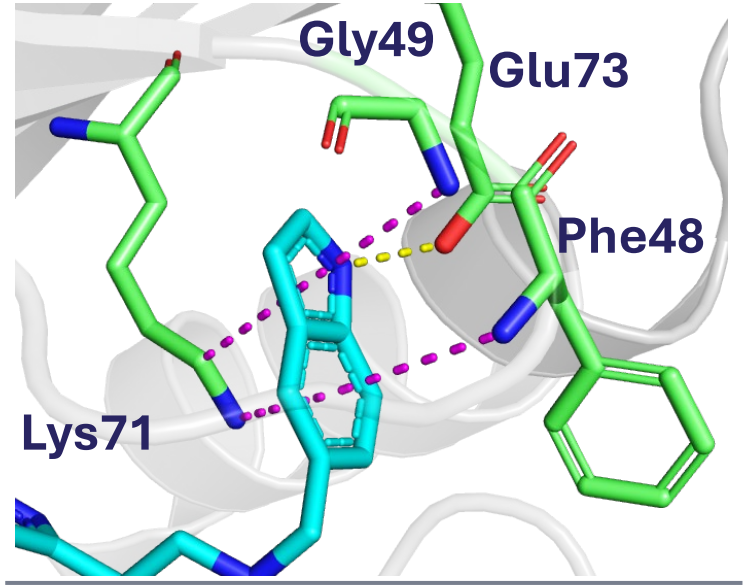
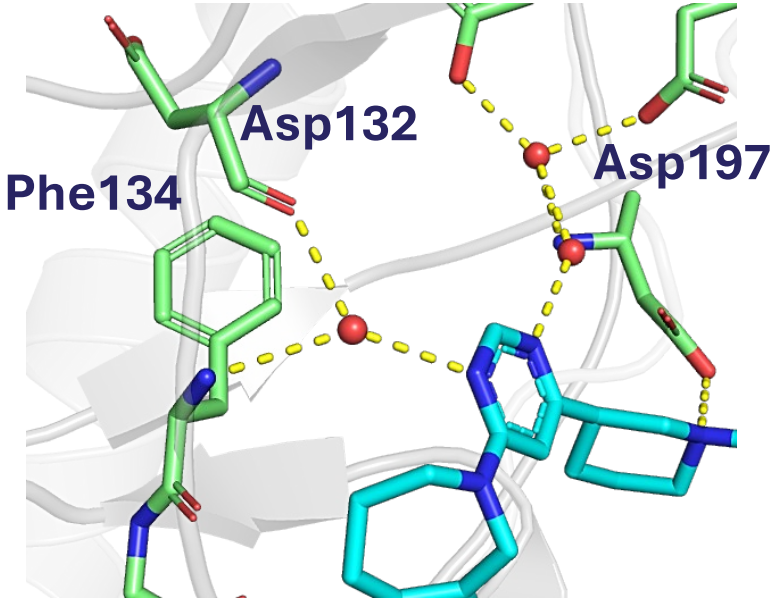
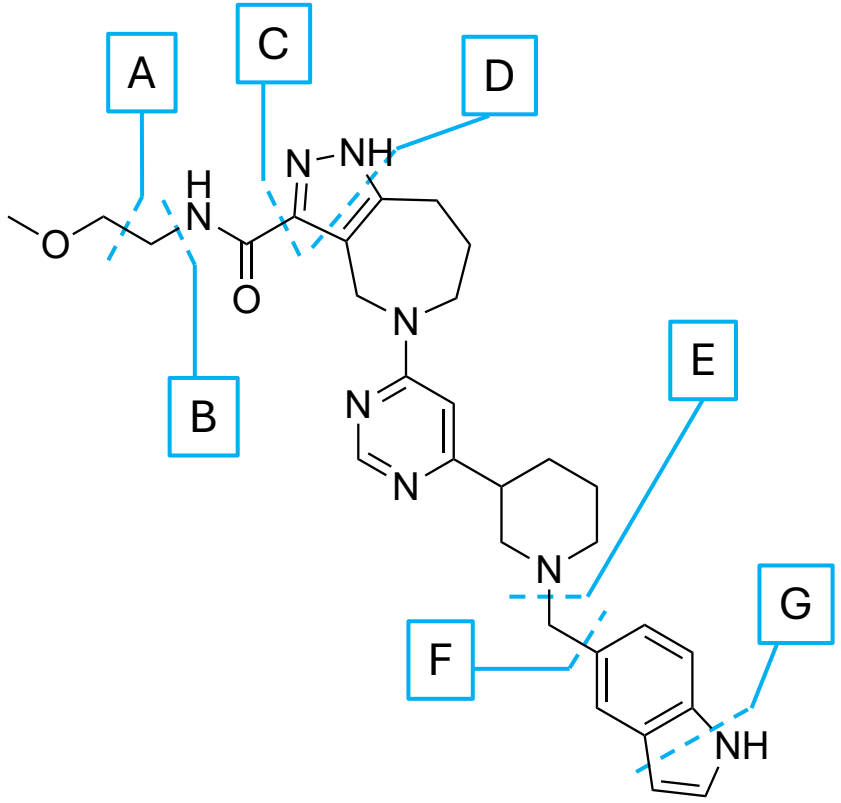
Reductive SAR identifies indole moiety as key driver of potency



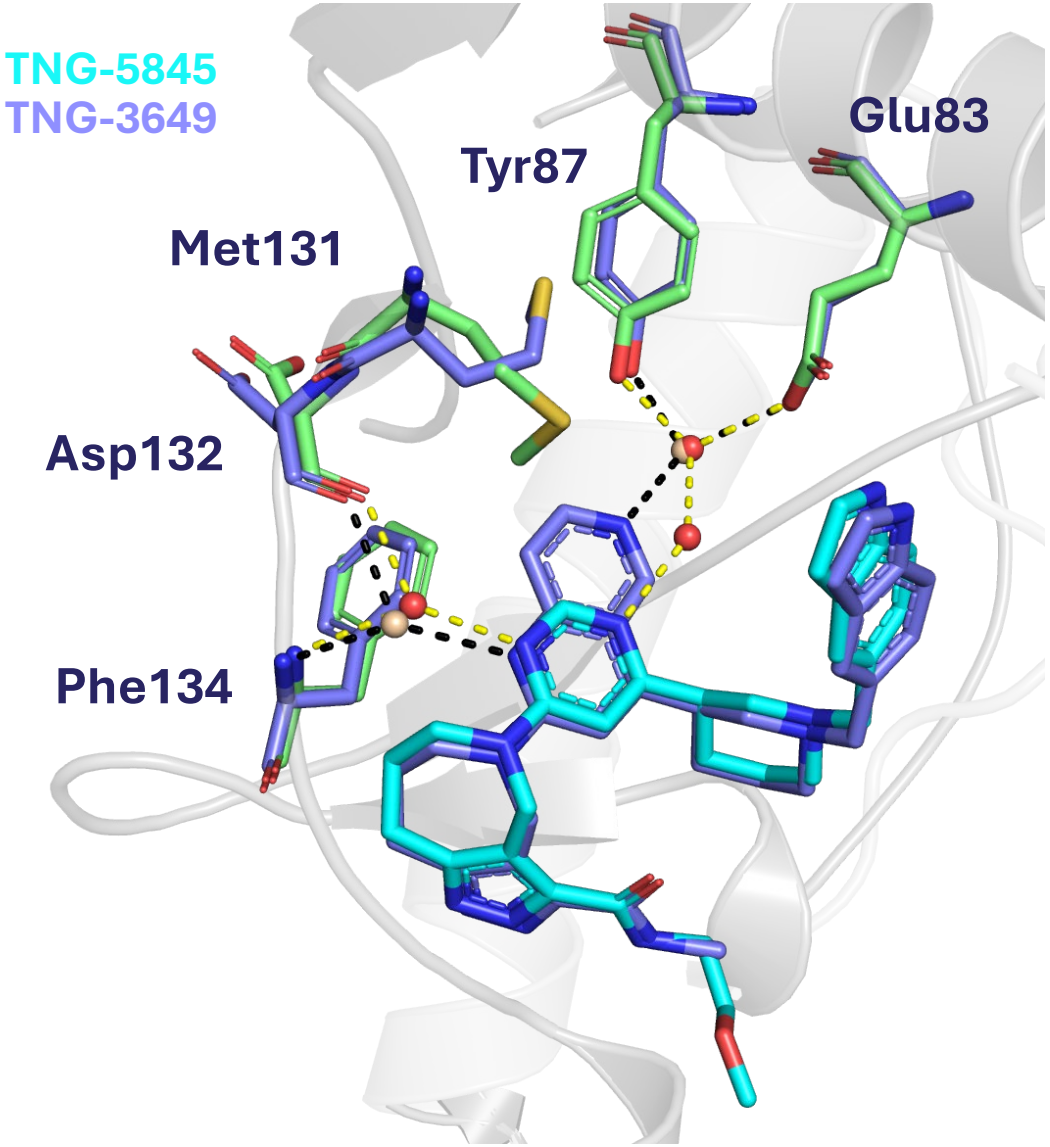


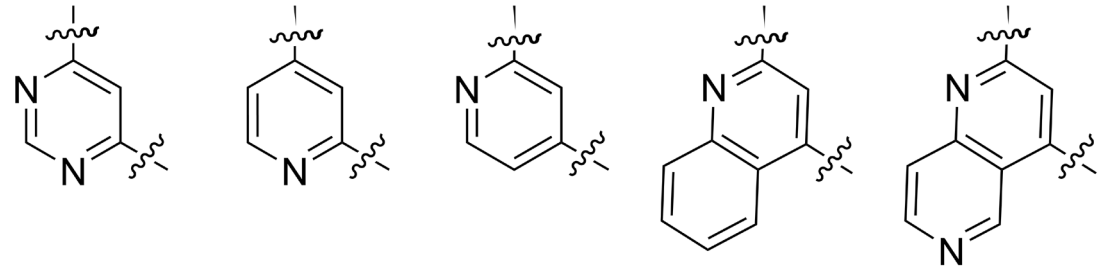
TNG-5845



	Fold decrease in potency
A	3
B	6
C	176
D	253
E	> 1300
F	> 1300
G	> 1300

Replacement of pyrimidine core with naphthyridine leads to improved potency





TNG-	**8840**	**6916**	**6915**	**2297**	**3649**
VRK1 IC_{50} (μM) ATP = K_m	0.075	0.95	0.27	0.33	< 0.006*
VRK1 IC_{50} (μM) ATP = 1 mM	0.314	12.3	2.9	1.44	< 0.002*
VRK2 IC_{50} (μM) ATP = 1 mM	> 30	-	-	2.05	0.162
VRK1 Cell IC_{50} (μM)	0.284	-	-	2.41	0.007

*below lower limit of assay

TANGO therapeutics

Summary

Covalent screen

- Revealed a novel Cys50 binding pocket and showed that potency is driven by warhead reactivity rather than binding affinity
- Established a validated framework for covalent inhibitor evaluation

Cell-based Vipergen DEL

- Identified a cell-active chemotype distinct from conventional hinge-binding and back-pocket kinase inhibitors
- Delivered a potent, selective VRK1 inhibitor with favorable physicochemical properties through early SAR

Acknowledgements

Biochemistry: Katarzyna B. Handing, Mu-Sen Liu, Brian McMillan, Wenhai Zhang, William D. Mallender

Discovery Biology: Kiera Vassallo, Alvin Lu, Julie Shields, Natasha Emmanuel, Kimberly J. Briggs, Robert Tjin Tham Sjin

Chemistry: Patrick McCarren, Brett Williams, Jon Come, Kevin Cottrell

Vipergen

Biortus

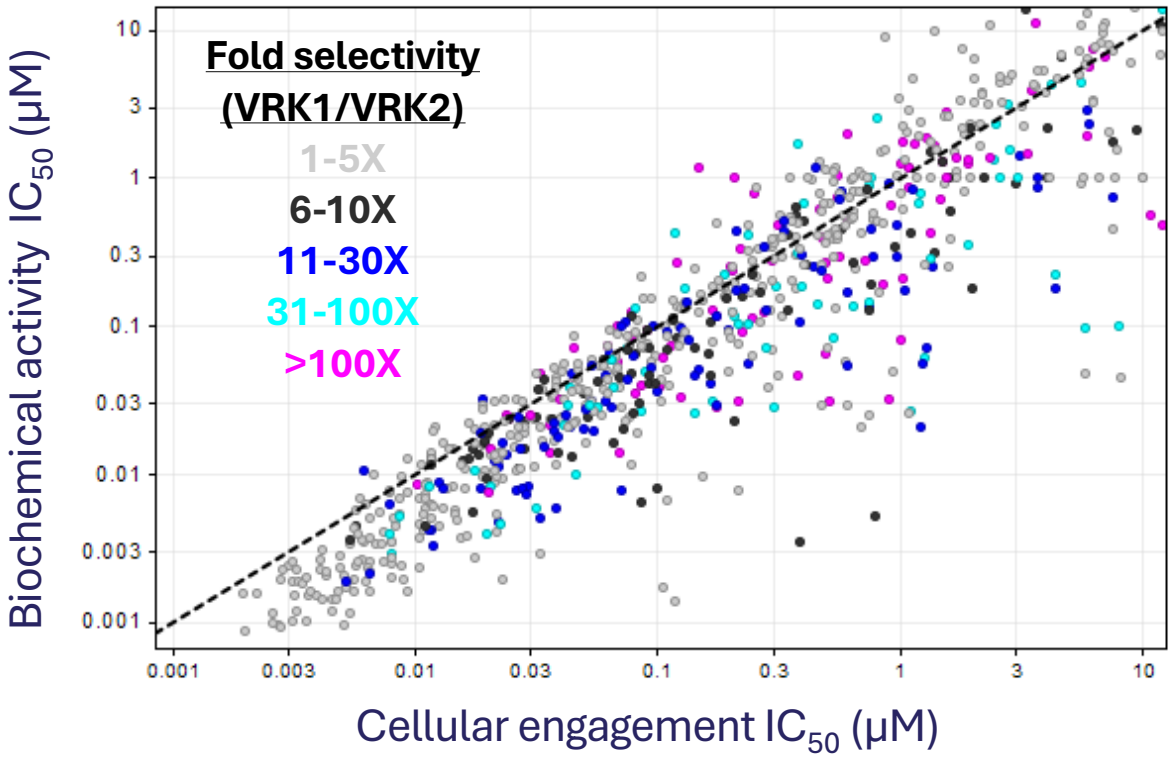
Wuxi AppTec

Enamine

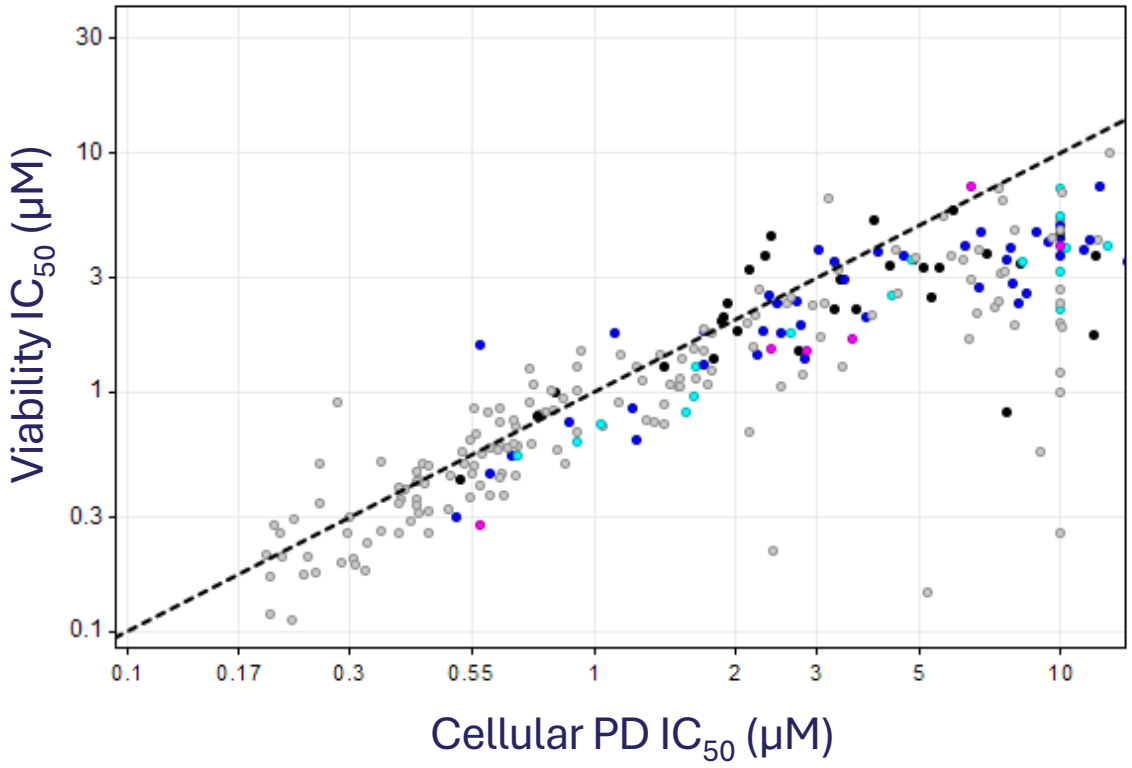
TANGO
therapeutics™

Strong VRK1 drug discovery enablement through assay concordance

VRK1 activity in biochemical assay correlates with cellular engagement



VRK1 target engagement predicts lethality in VRK2-Low Cells



TANGO therapeutics