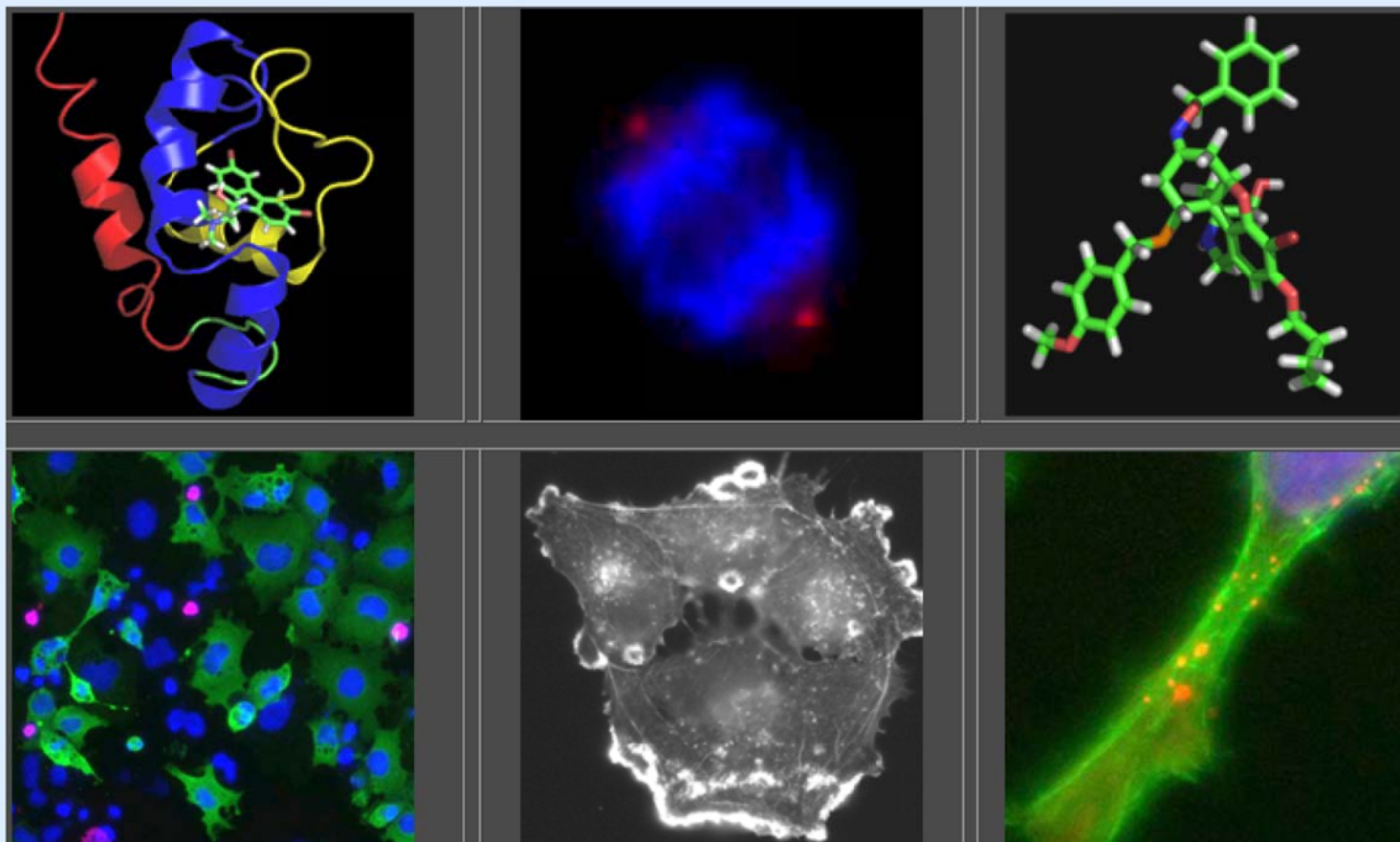
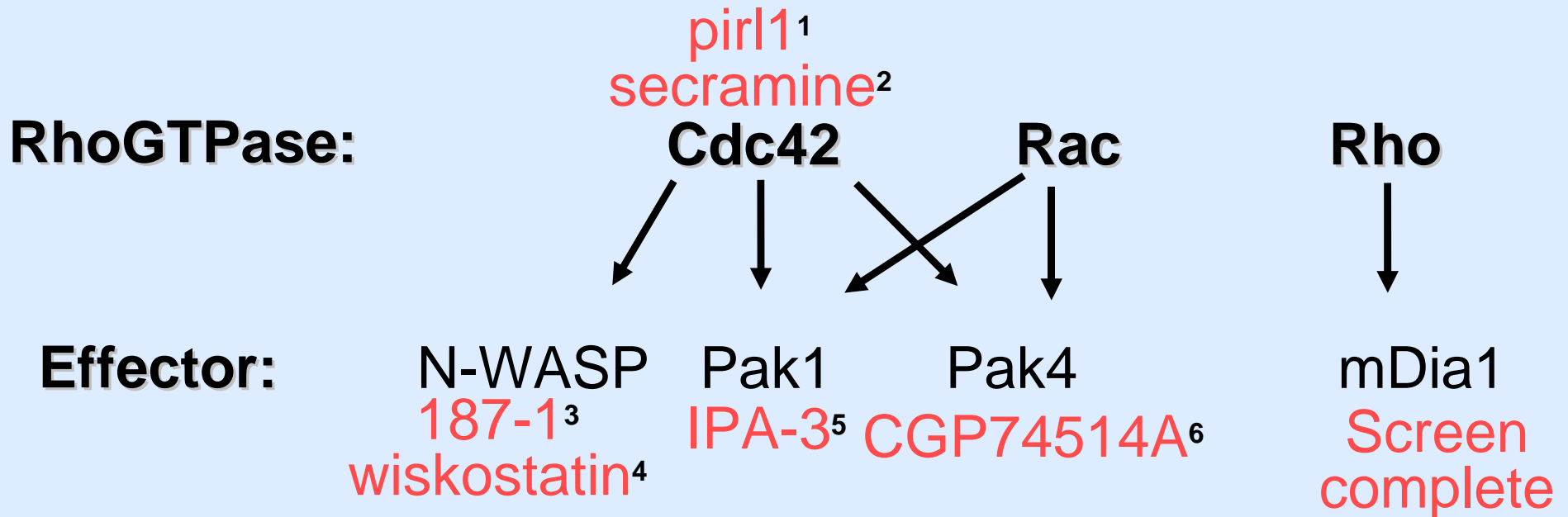


Chemical genetic approaches to inhibitor discovery

Jeff Peterson, Ph.D.



Chemical inhibitors of Rho GTPase signaling



¹ *Chem. Biol.* **13**:443-452, 2006

² *Nat. Chem. Biol.* **2**:39-46, 2006

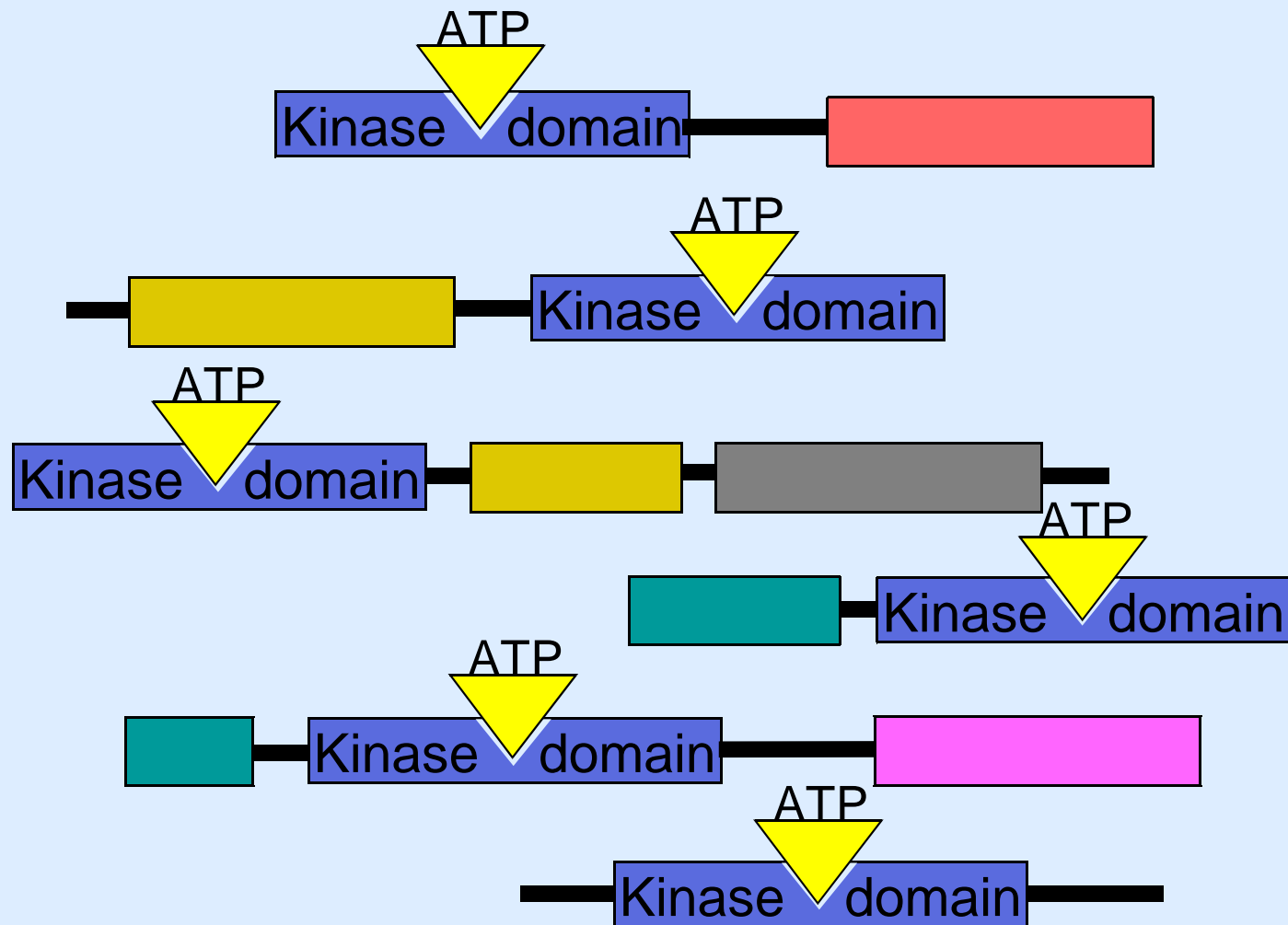
³ *Proc. Natl. Acad. Sci. USA* **98**:10624-10629, 2001

⁴ *Nat. Struct. Mol. Biol.* **11**:747-755, 2004

⁵ *Chem. Biol.* **15**:322-331, 2008

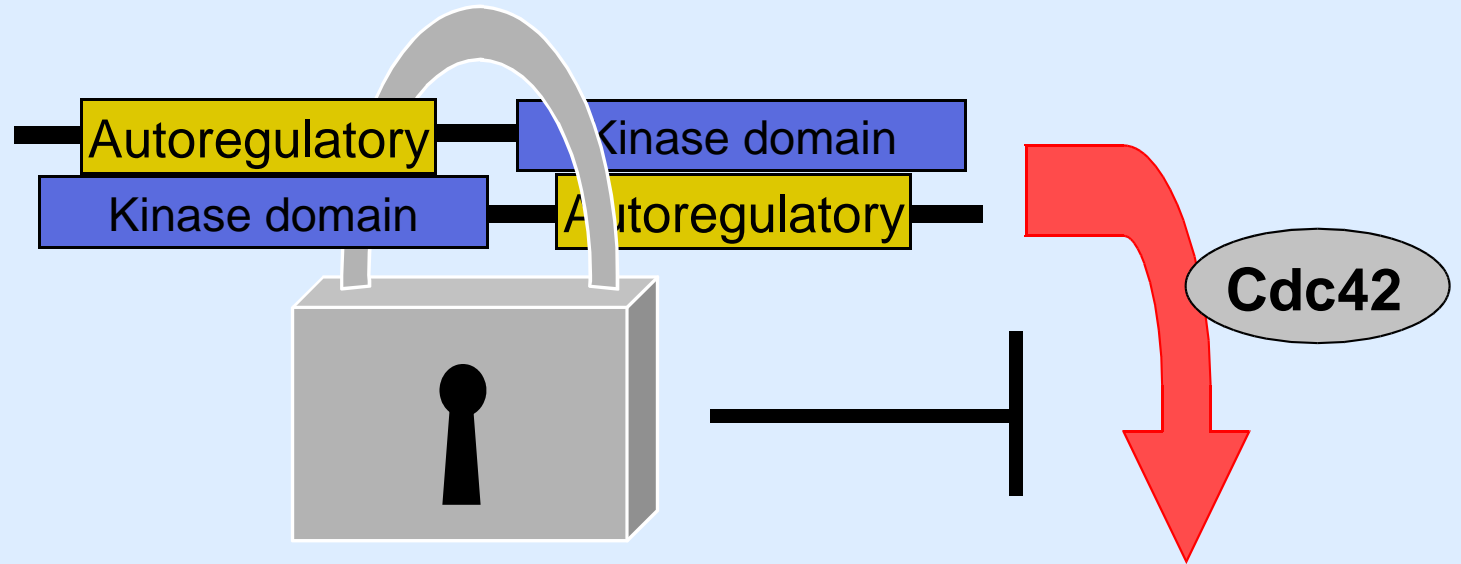
⁶ *Structure* **15**:201-213, 2007

Kinase active sites are conserved
but regulatory domains may be unique

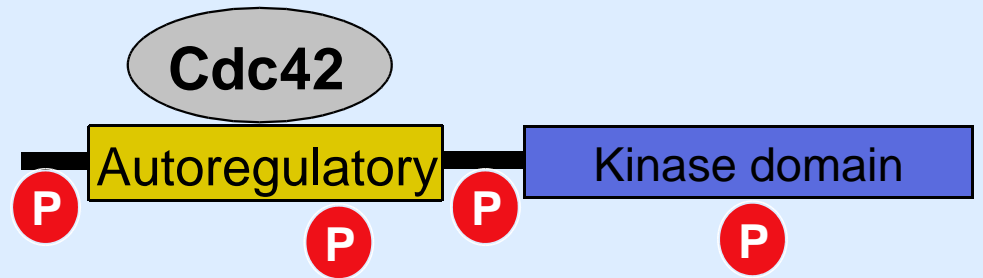


Pak1 regulation

Inhibited

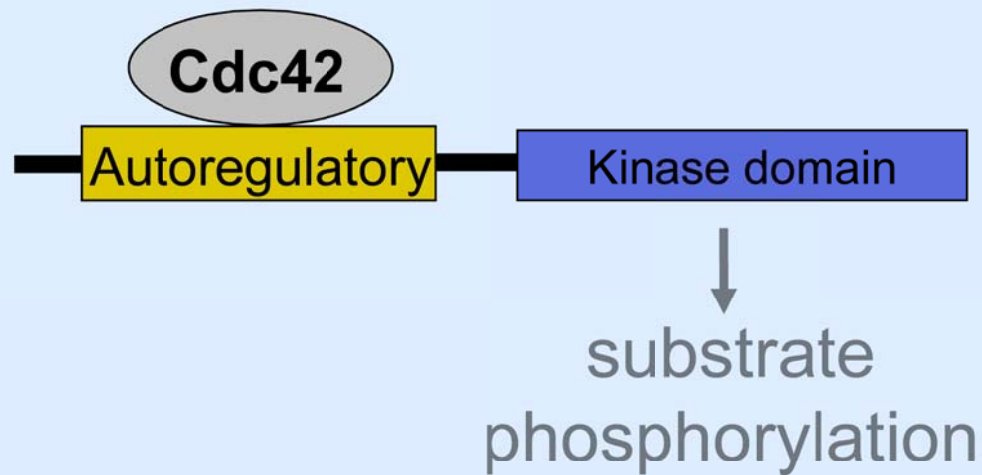


Active

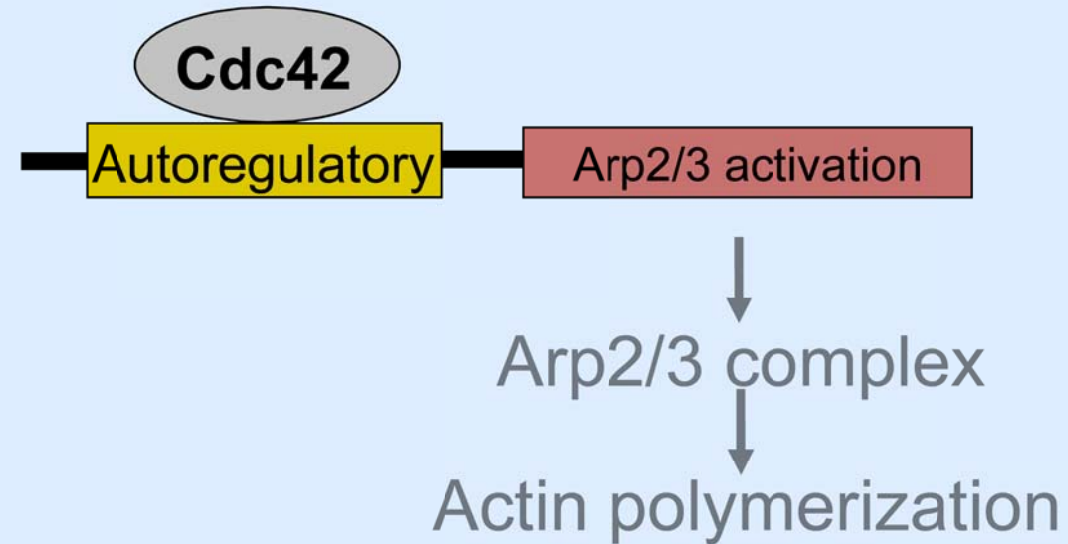


Pak1 & WASP share a conserved autoregulatory domain

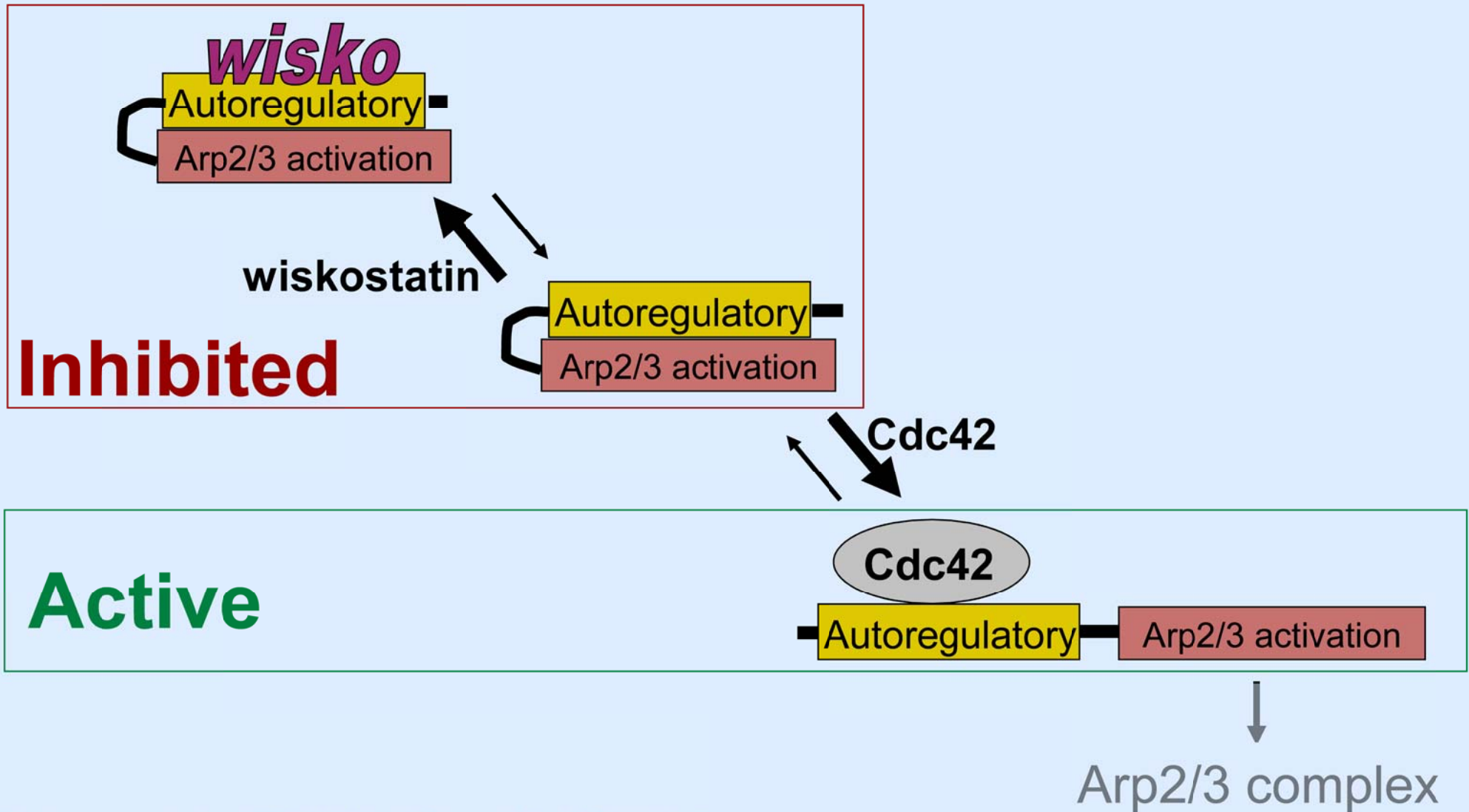
Pak1



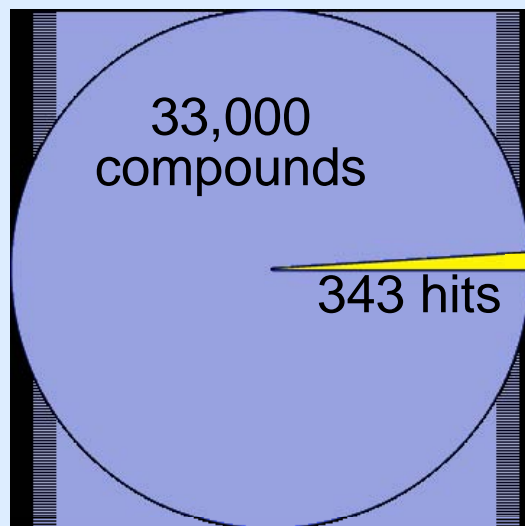
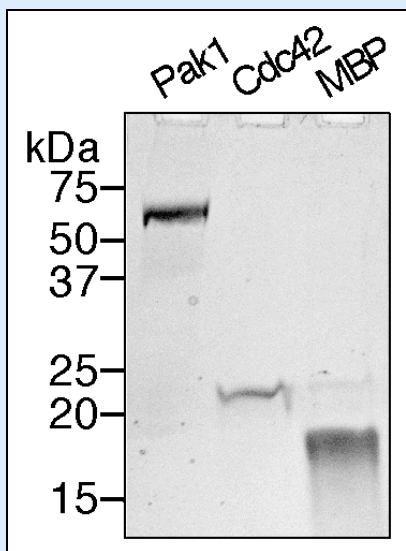
WASP



Wiskostatin stabilizes the autoinhibited conformation of WASP



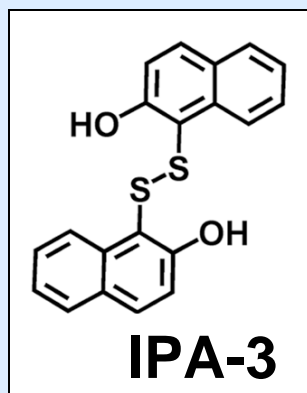
A screen for Inhibitors of Pak1 Activation)



Retest at 1 mM ATP

32 IPAs

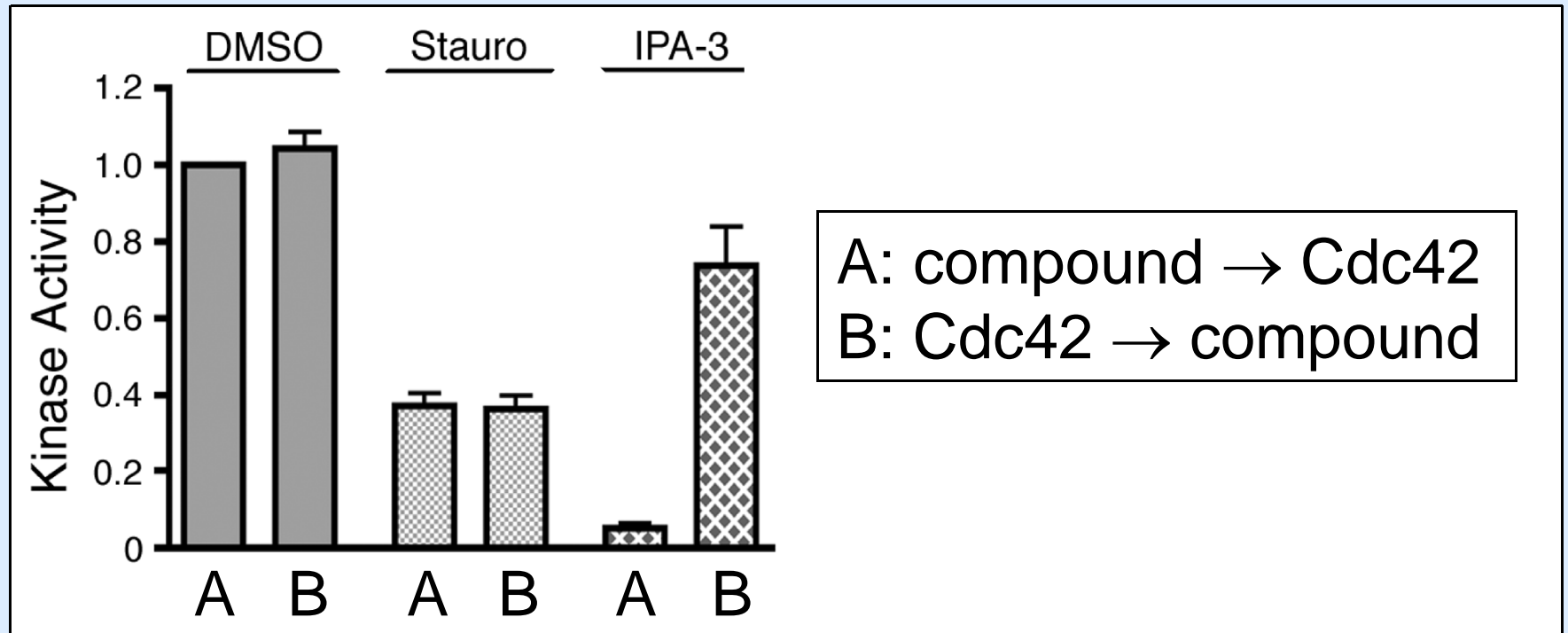
311 Inactive



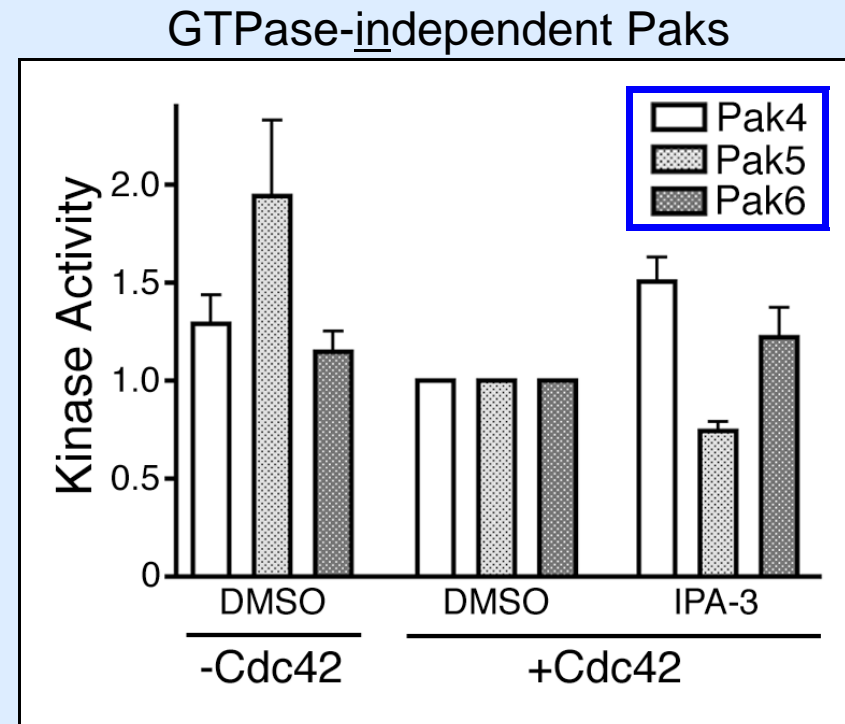
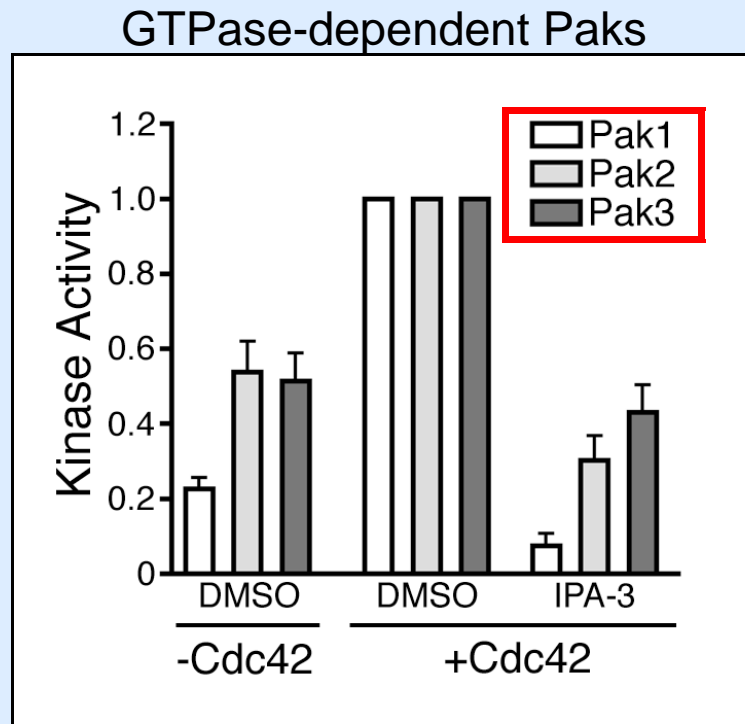
$IC_{50} = 2.5 \mu M$

Jami Fukui & Sean Deacon & Sandy Beeser

IPA-3 targets a Pak1 activation intermediate



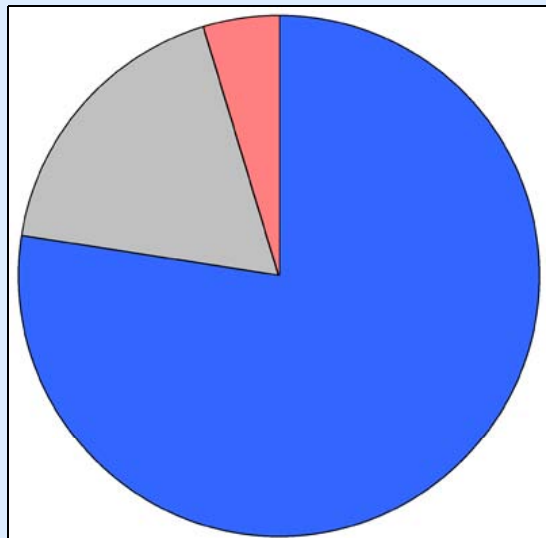
IPA-3 inhibits GTPase-dependent Pak isoforms



Adapted from Manning et al. (2002) Science 298: 1912.

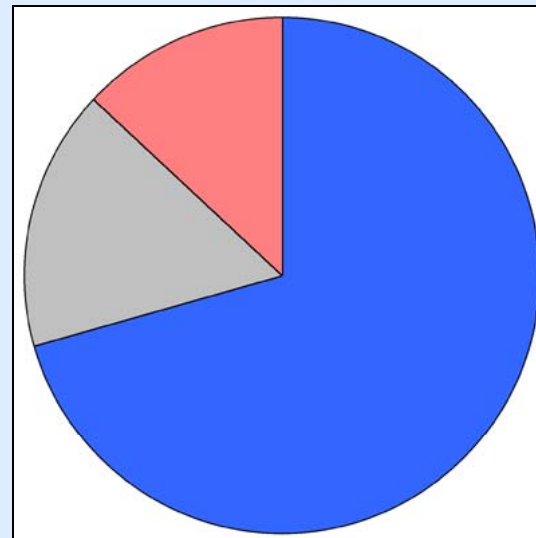
Kinase profiling shows IPA-3 is a highly selective kinase inhibitor

IPA-3



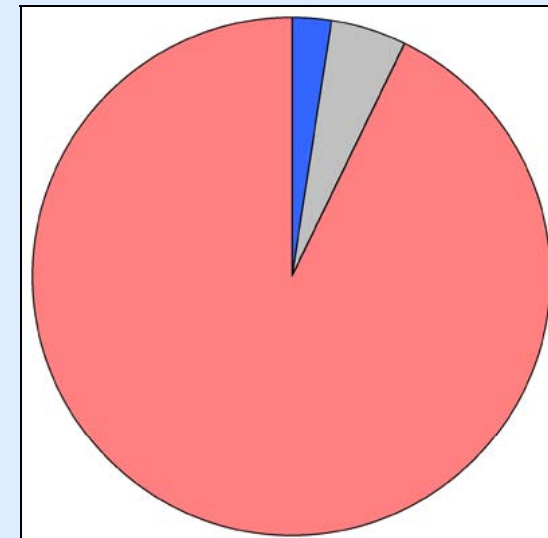
n=214 kinases

Gleevec

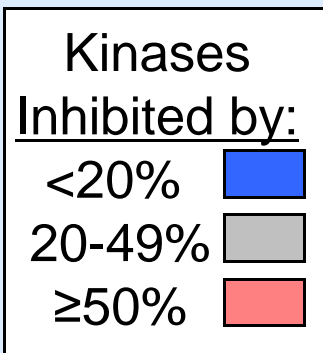


n=193 kinases

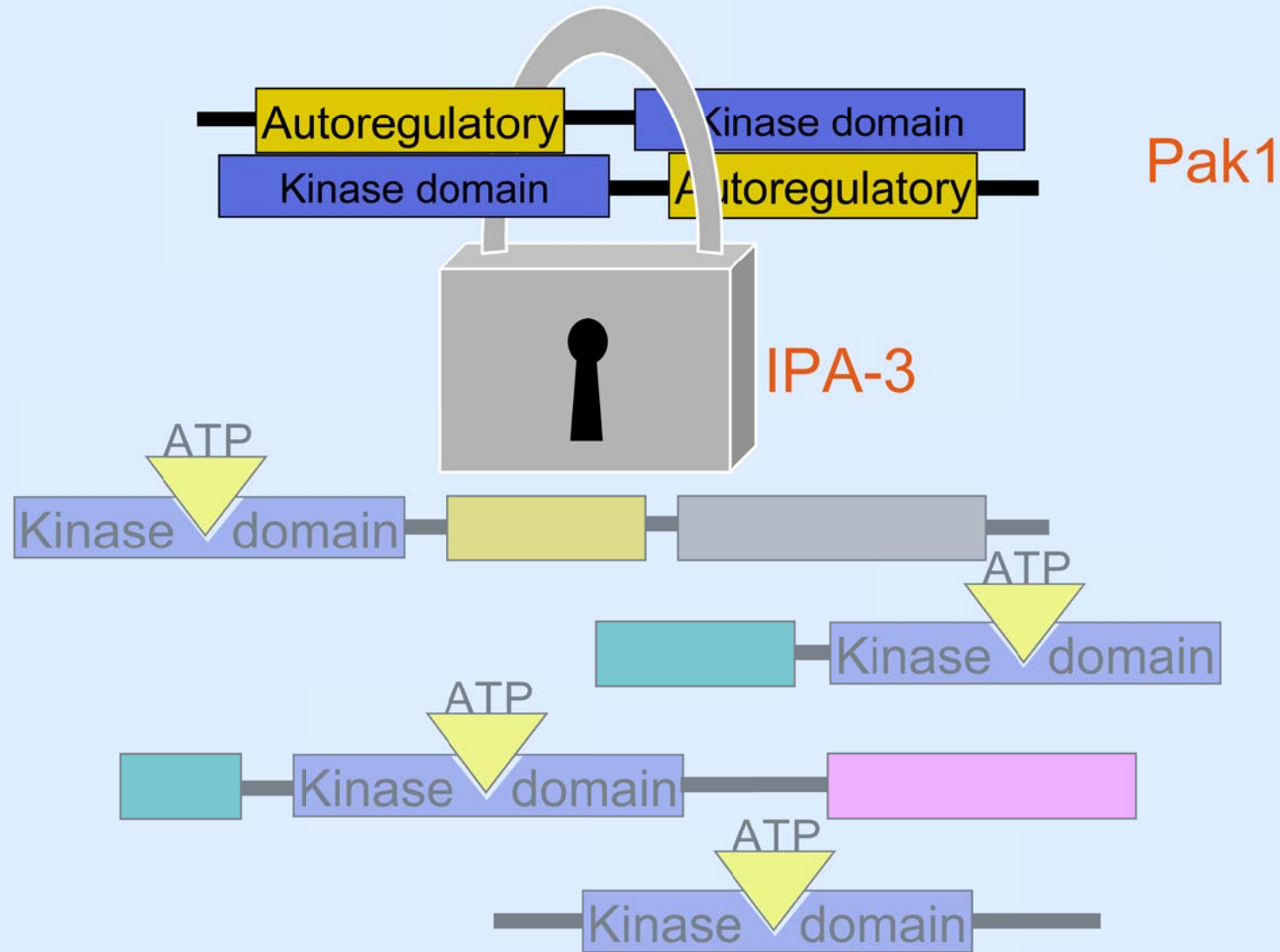
Staurosporine



n=193 kinases

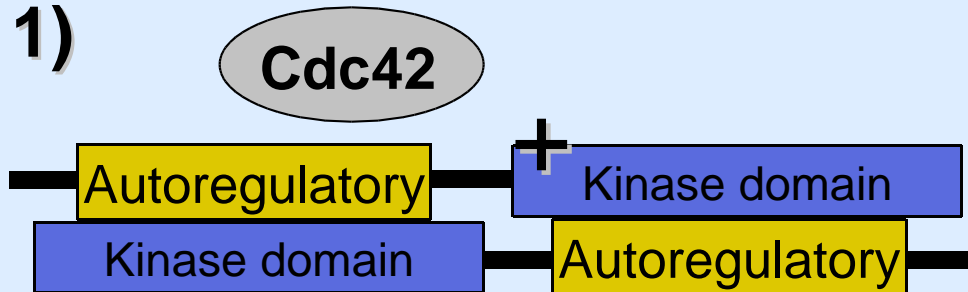


Autoregulatory domains may offer alternative inhibitor targets with greater kinase selectivity

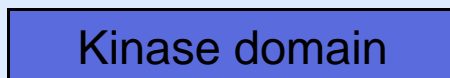


A dual screen for allosteric inhibitors

1)



2)



Inhibitor active?

-	+	+
-	+	-

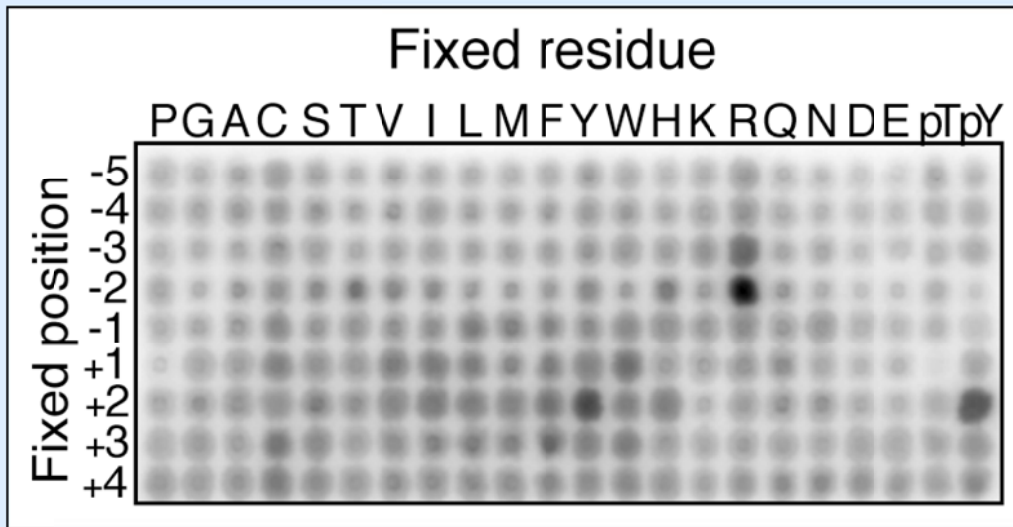
Not an inhibitor

Active site inhibitor or non-specific

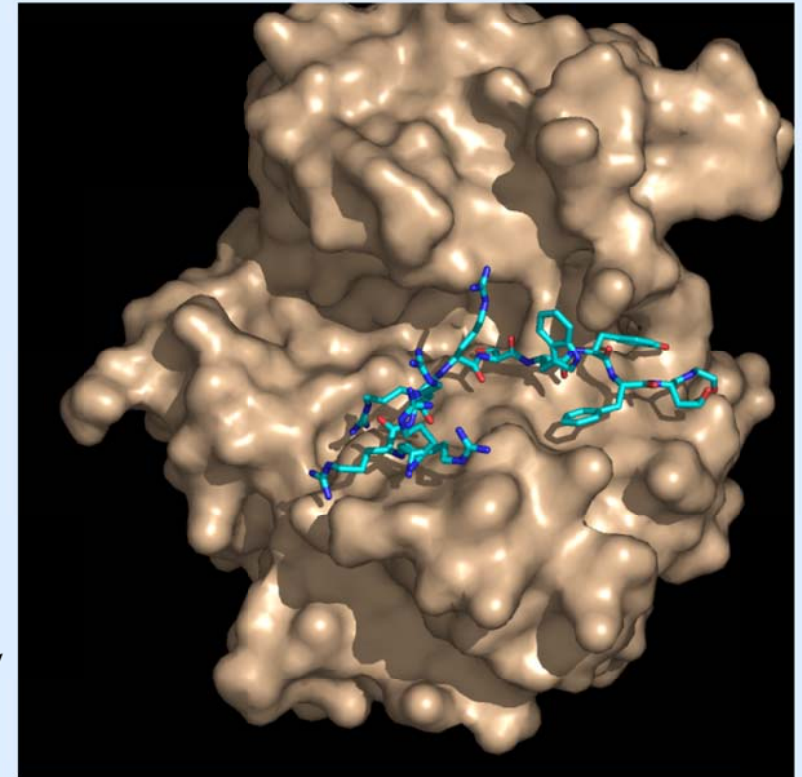
Putative allosteric inhibitor

Interpretation:

Pak substrate recognition



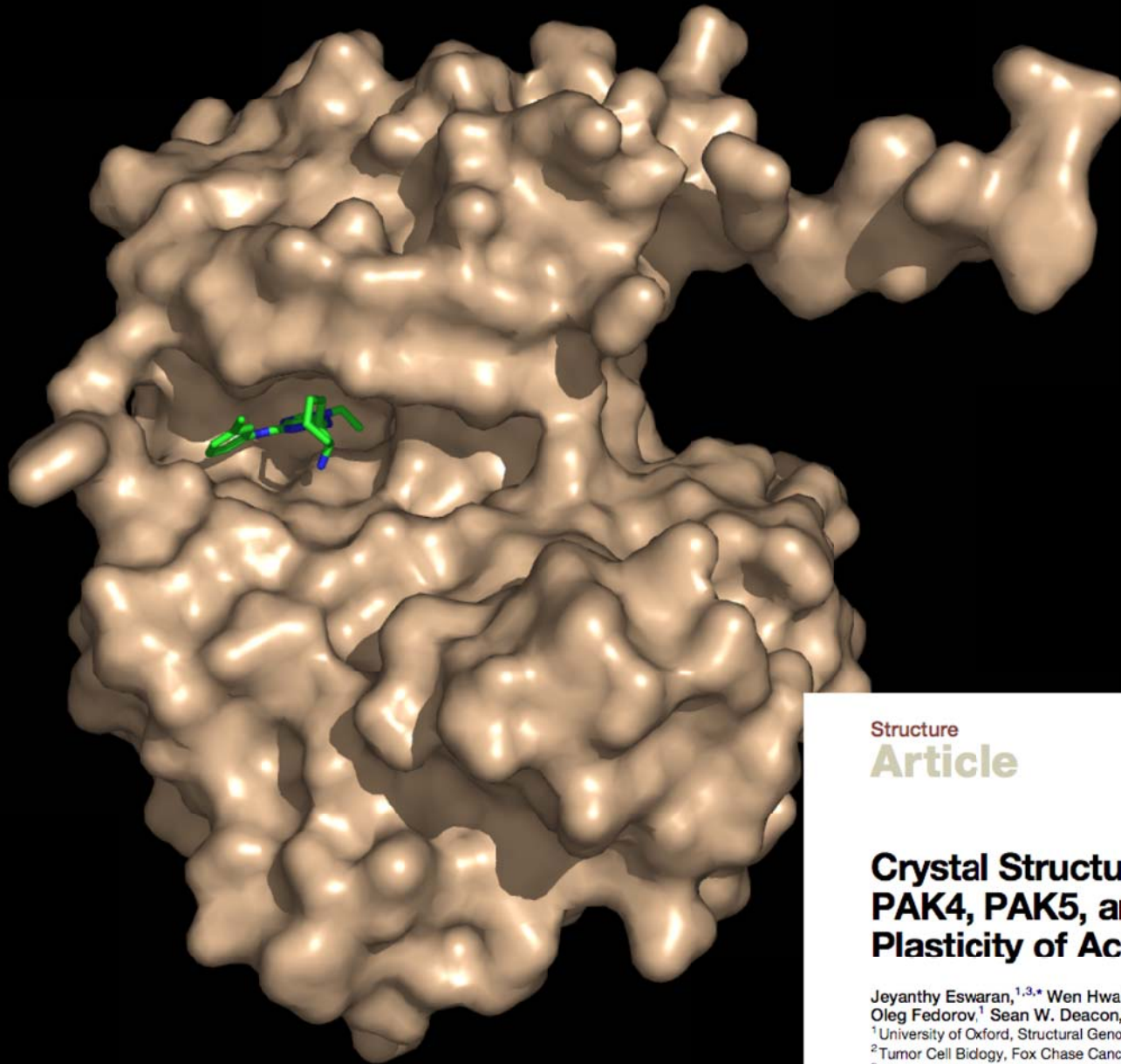
Rennefahrt et al., (2007) J Biol Chem 282:15667



Structural Genomics Consortium,
Oxford, unpublished

Optimal substrate: GRRRRRRSWYFGGGK

Pak5 bound to inhibitor CGP74514A



Structure
Article

Cell
PRESS

Crystal Structures of the p21-Activated Kinases PAK4, PAK5, and PAK6 Reveal Catalytic Domain Plasticity of Active Group II PAKs

Jeyanthi Eswaran,^{1,3,*} Wen Hwa Lee,^{1,3} Judit É. Debreczeni,¹ Panagis Filippakopoulos,¹ Andrew Turnbull,¹
Oleg Fedorov,¹ Sean W. Deacon,² Jeffrey R. Peterson,² and Stefan Knapp^{1,*}

¹University of Oxford, Structural Genomics Consortium, Botnar Research Centre, Oxford OX3 7LD, United Kingdom

²Tumor Cell Biology, Fox Chase Cancer Center, 333 Cottman Avenue, Philadelphia, PA 19111, USA

³These authors contributed equally to this work.

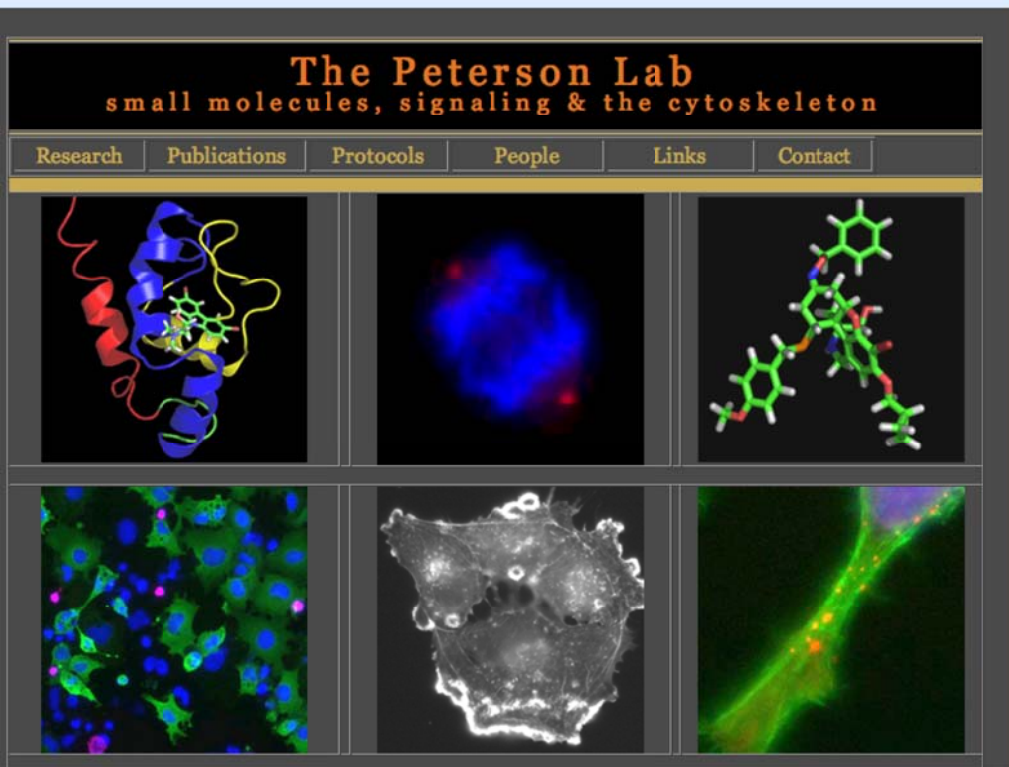
*Correspondence: jeyanthi.eswaran@sgc.ox.ac.uk (J.E.), stefan.knapp@sgc.ox.ac.uk (S.K.)

DOI 10.1016/j.scr.2007.01.001

Key Points

- Autoregulatory domains may offer alternative targets for inhibitors
- A screening method to identify such inhibitors
- Biochemical and structural data on binding of substrates and inhibitors to Paks

Need more information?



Website:

<http://labs.fccc.edu/peterson/>

Poster during lunch

Jeffrey.peterson@fccc.edu

Collaborators

- Jonathan Chernoff (FCCC)
- Benjamin Turk (Yale)
- Stefan Knapp (SGC Oxford)

Relevant Publications

Wiskostatin - *Nat. Struct. Mol. Biol.* **11**:747-755, 2004

IPA-3 - *Chem. Biol.* **15**:322-331, 2008

Review: Autoinhibited proteins as drug targets

- *J. Cell. Biochem.* **93**:68-73, 2004

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- DoD Neurofibromatosis

Research Program

- AACR
- Pennsylvania Tobacco Settlement