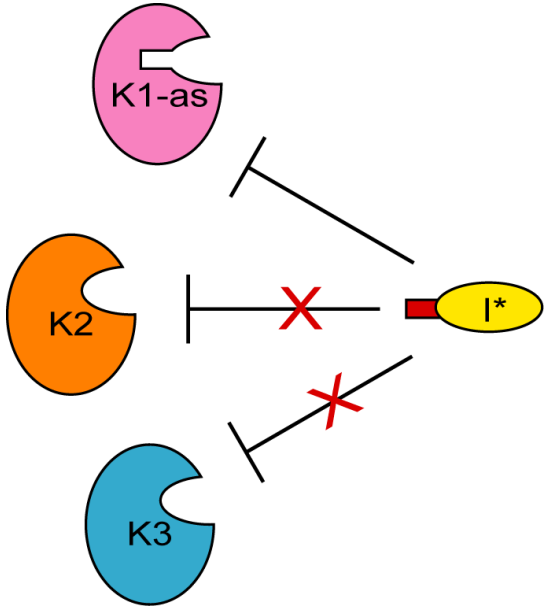
# *Kinase Chemical Genetics*




## *New Tools for Old Problems*

MacMillan Group Meeting

July 1st, 2009

Alexander Warkentin

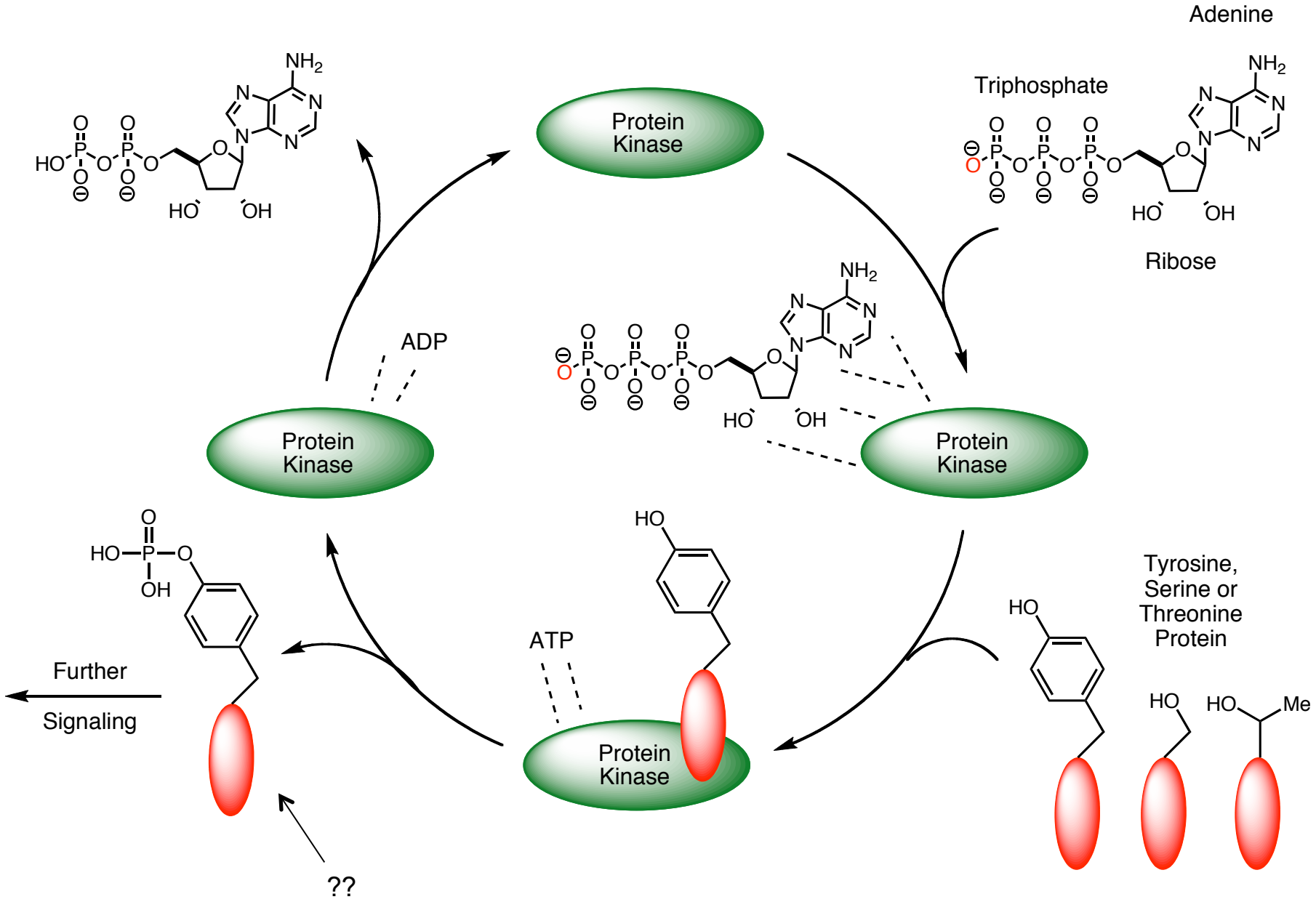
# *Outline*

- Background biology notes
- Inspiration for kinase research
- Chemical genetics: inhibition, benefits for signaling subtleties
- Chemical genetics: A*TP analogues, benefits for direct phosphoprotein substrate detection
- *in vivo* example

*Kevan M. Shokat*

- 1991 Ph.D. Chemistry, University of California, Berkeley
  - Advisor: P. G. Schultz; New Routes to Catalytic Antibodies
- 92' – 94' Post-doctoral scholar, Stanford University
  - Advisor: C. C. Goodnow; Immune self tolerence in transgenic mice
- 94' – 98' Assistant Professor of Chemistry and Molecular Biology, Princeton University
- 98' – 99' Associate Professor of Chemistry and Molecular Biology, Princeton University
- 99' – 01' Associate Professor of Chemistry, Berkeley; Pharmacology, UCSF
- 01' Professor of Chemistry, Berkeley; Pharmacology, UCSF
- 05' Investigator, Howard Hughes Medical Institute

# *What a Protein Kinase Does: Basin Catalytic Cycle*



## *Kinases Covered*

- CDK2 – Cyclin-dependent kinase: critical for cell cycle
  - cdc28 – yeast version of CDK2: used for easier analysis
- bcr, abl – Together as a fusion protein kinase: leukemia
  - PYK2 – Similar to bcr/abl in mode of inhibition
- v-Src – First known kinase (Krebs, 1959): best studied
- EGFR – Epithelial Growth Factor Receptor: breast, lung cancer
- Aurora B, Hck, Ire1, p110
- CAMKII – Hippocampal long-term memory formation

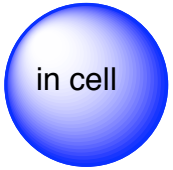
Misregulation implicated in metastasis

# *Background: Medium of Study*

- *in vitro*: Isolated enzyme or other biomolecule.
  - First line of analysis; no interference or off target effects
  - Relatively fast; requires less than one milligram of small molecule

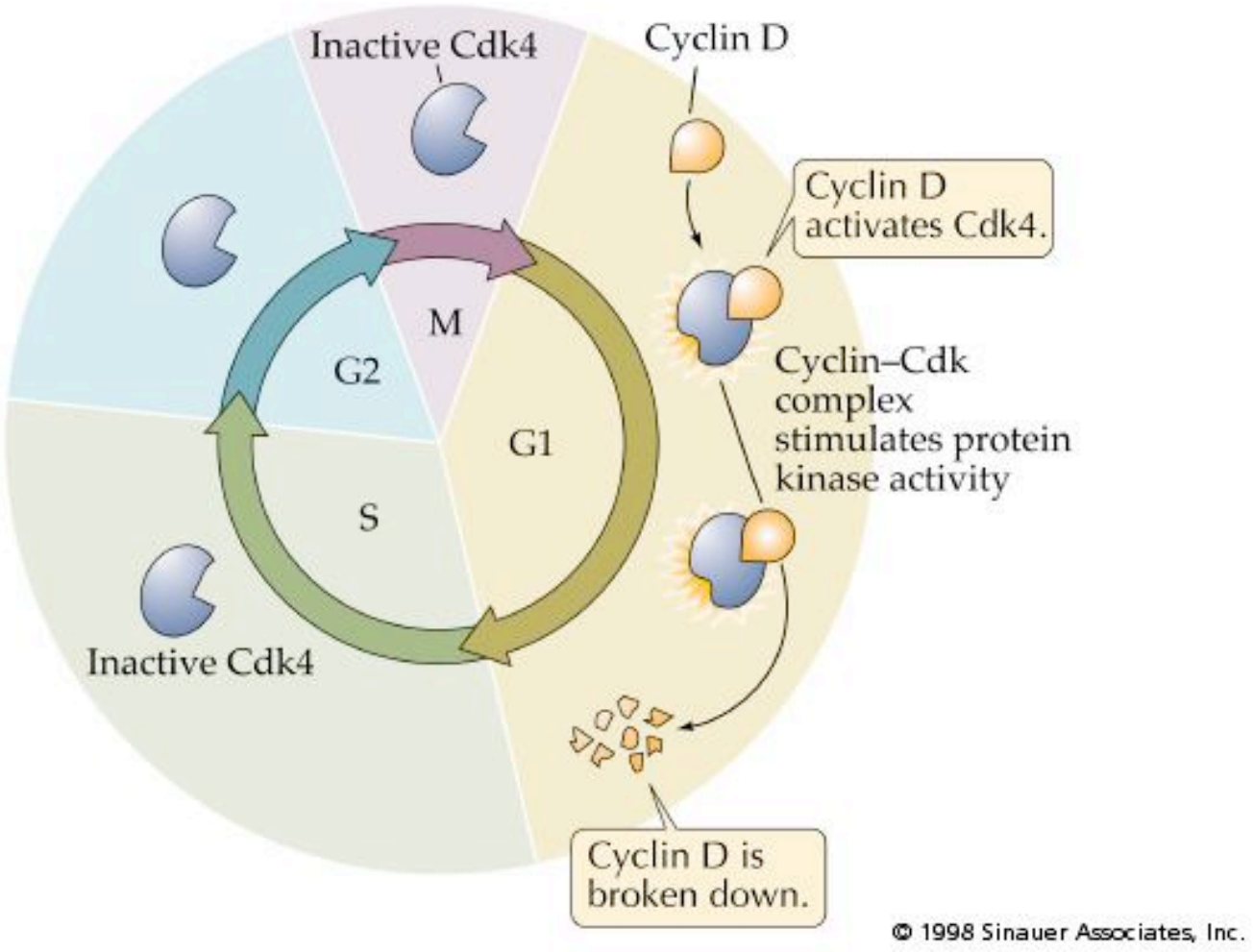

- *in lysate*: study occurs in cell-free environment in medium with other proteins
  - Not used very often

- In cell: mostly yeast cells
  - A non-trivial level of complexity already

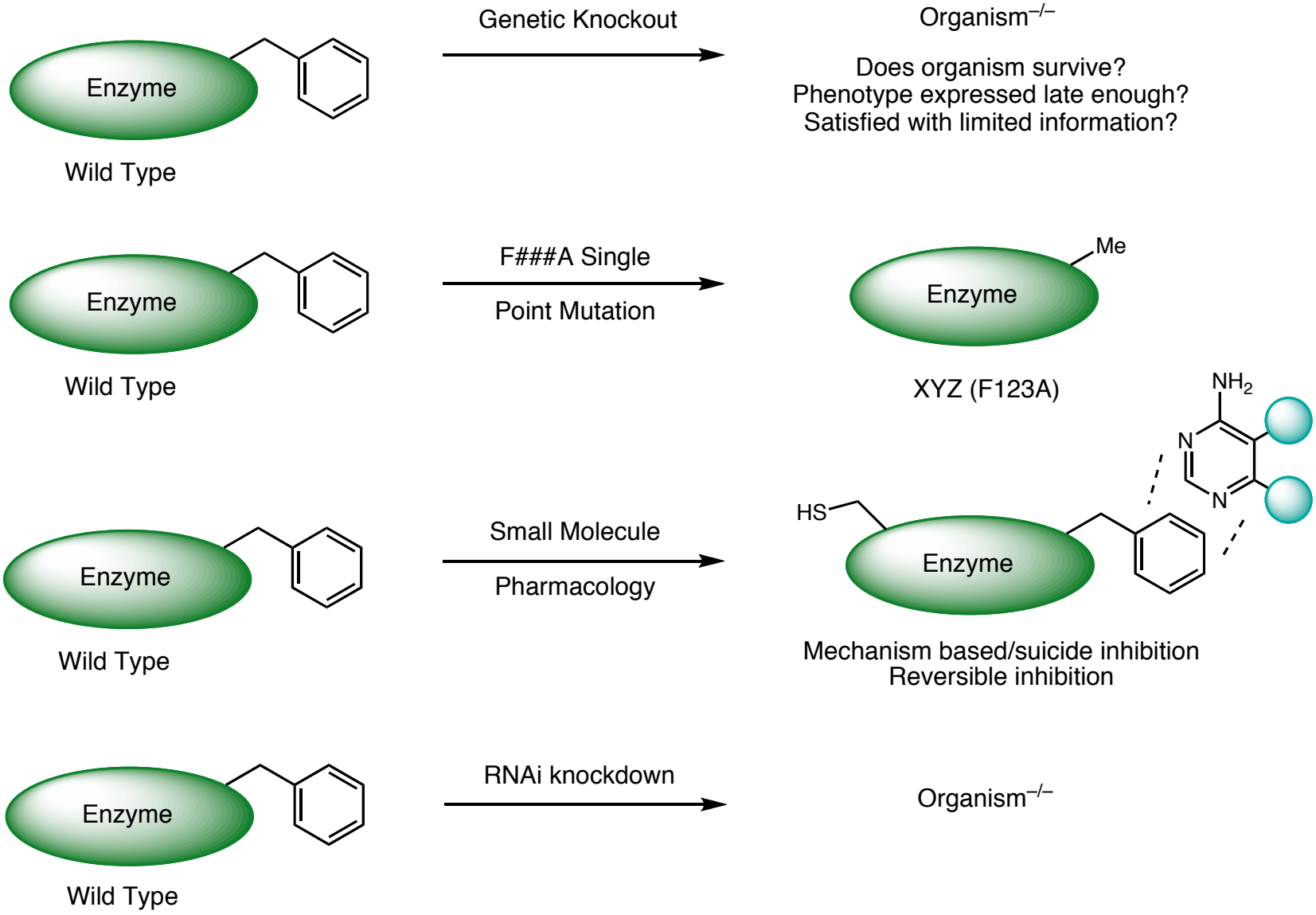



- *in vivo*: Usually starts in the mouse
  - Mouse genome close to humans
  - Knockout experiments risk aborting embryogenesis; pharmacology risks off-target effects

*Background: Cell Cycle*



# *Background: Experiments and Questions*



# *Background: Experiments and Questions*

- Upregulative compensation?
- Know "that", not "how".

Genetic Knockout →

Organism$^{-/-}$

Does organism survive?
Phenotype expressed late enough?
Satisfied with limited information?

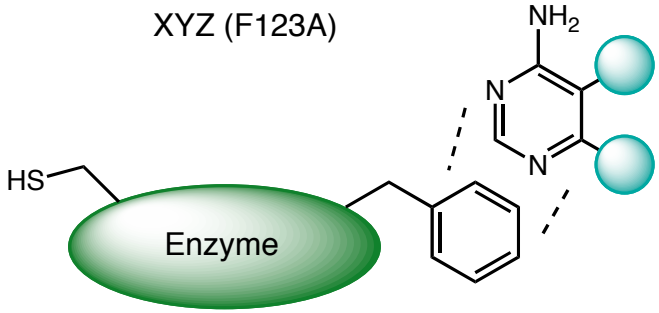
- Better for organismal survival
- Turns enzyme off?
- Still don't know targets

F###A Single
Point Mutation →


XYZ (F123A)

- Binding selective for fewest targets?
- Direct drug application

Small Molecule
Pharmacology →



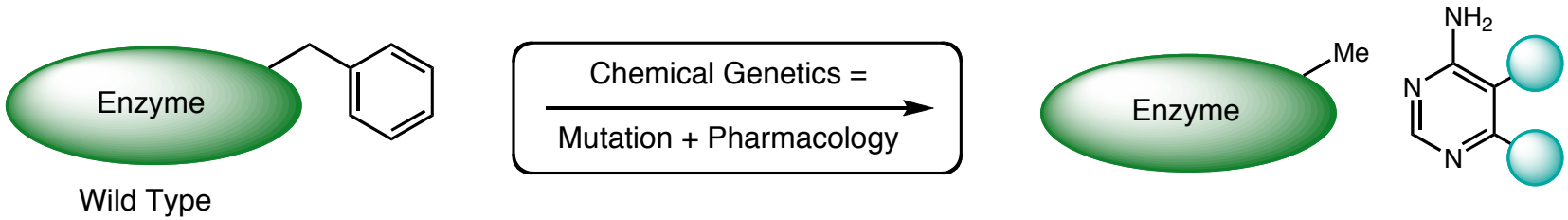

Mechanism based/suicide inhibition
Reversible inhibition

- Organism survives embryogenesis
- Inject double strand RNA
- Cell destroys its own enzyme
- Direct phenotypic response
- Knockdown a mild knockout
- Still don't know enzyme targets

RNAi knockdown →

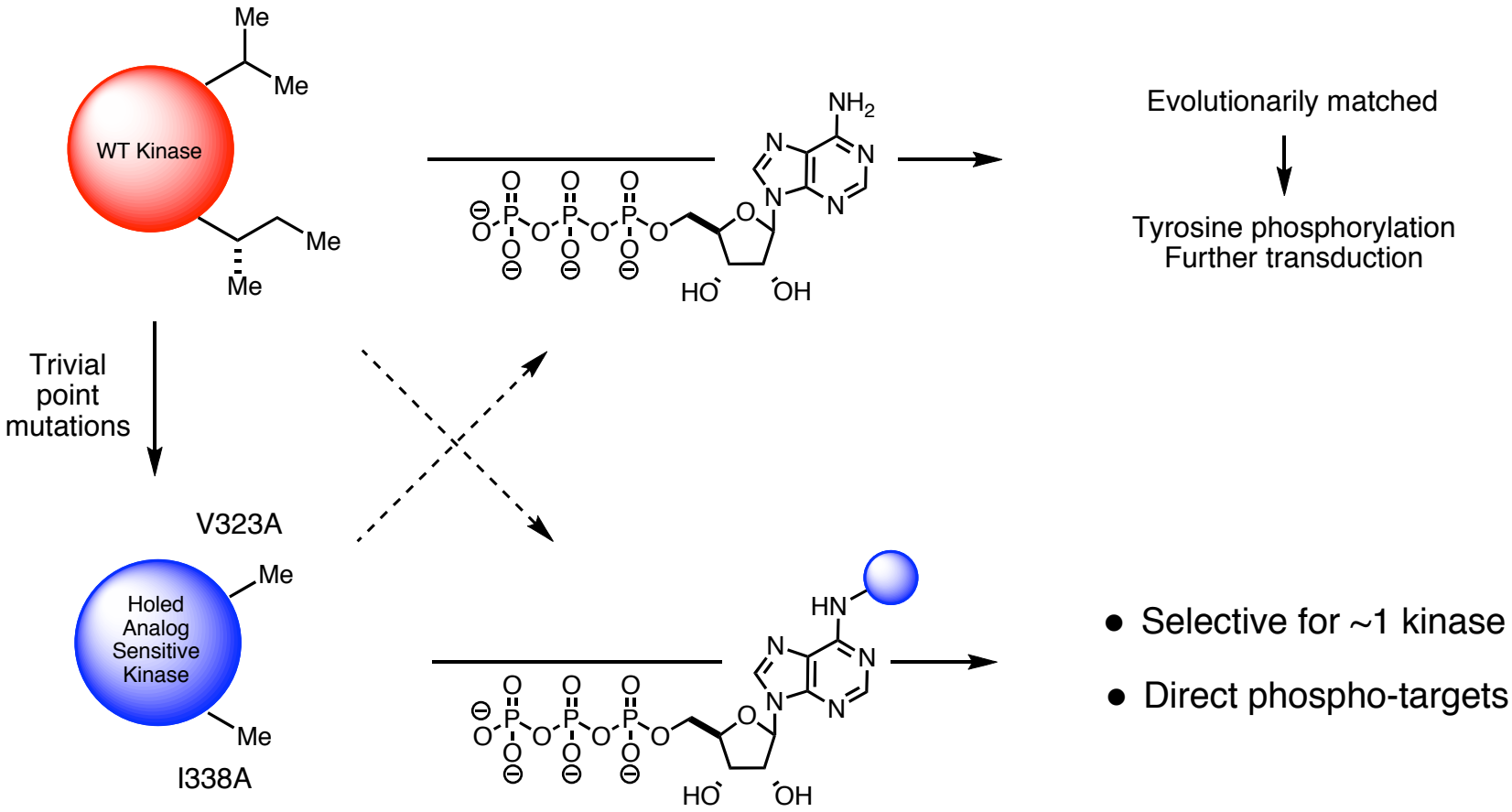
Organism$^{-/-}$

# *A New Experiment Combining Genetics and Pharmacology*




- Mutate only one kinase
- Match mutation with inhibitor
- Kinase specific information

# *A New Experiment Combining Genetics and Pharmacology*




- Selective for ~1 kinase
- Direct phospho-targets

# *Gleevec (Imatinib) Heralded as the "Magic Bullet"*

- Gleevec treats Chronic Myelogenous Leukemia (CML)

- At $32,000 per year for a 400 mg per day dose, cited as a justifiably high cost for pharmaceutical innovation

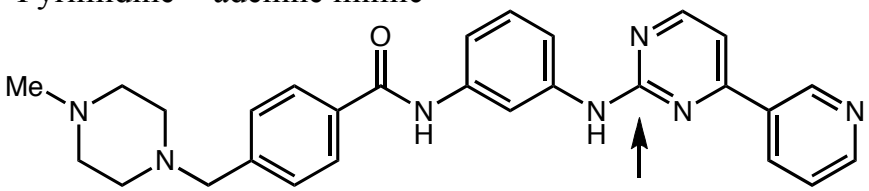
- Novartis challenges Indian patent law: Madras High Court rejects claim

- Sun Pharmaceuticals Industries Ltd. also challenges Novartis' US patent validity, which would set a decisive international precedent given the relative looseness of US patent law

- Novartis wins: 1st world innovation saved; Sun wins: 3rd world affords drugs


April 21st, 2001

Pyrimidine = adenine mimic




Gleevec (Imatinib)

# *(Gleevec) Imatinib: Mode of Action*

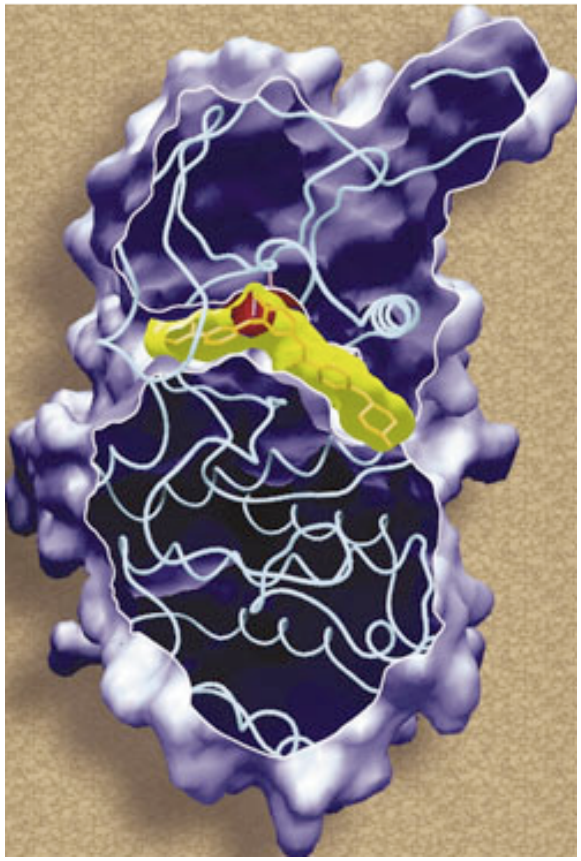
- Gleevec treats Chronic Myelogenous Leukemia (CML) and has been approved for gastrointestinal and other malignancies

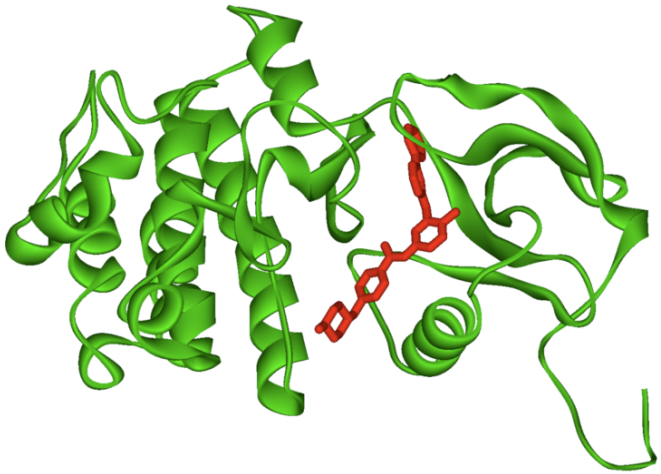
- CML results from chromosomal mutation that effects translocation of the bcr and c-abl-encoding genes, the resulting fusion being termed the Philadelphia chromosome or bcr-abl oncogene

- Expression of the bcr-abl fusion protein results in a myeloid cell line which is termed "growth factor independent for proliferation."

- This thwarts apoptosis and leads to metastases

- Treatable with bone marrow transplantation but only for 20% of patients due to age or compatibility

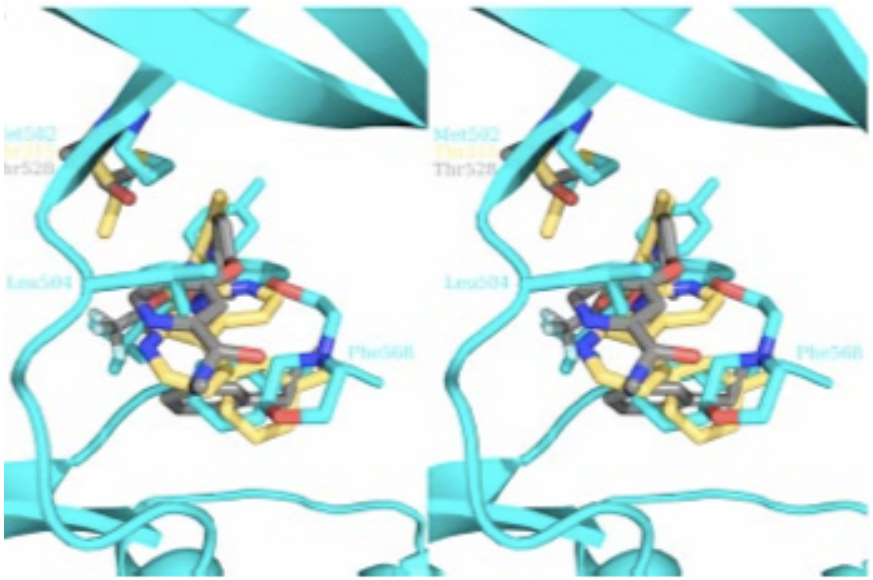




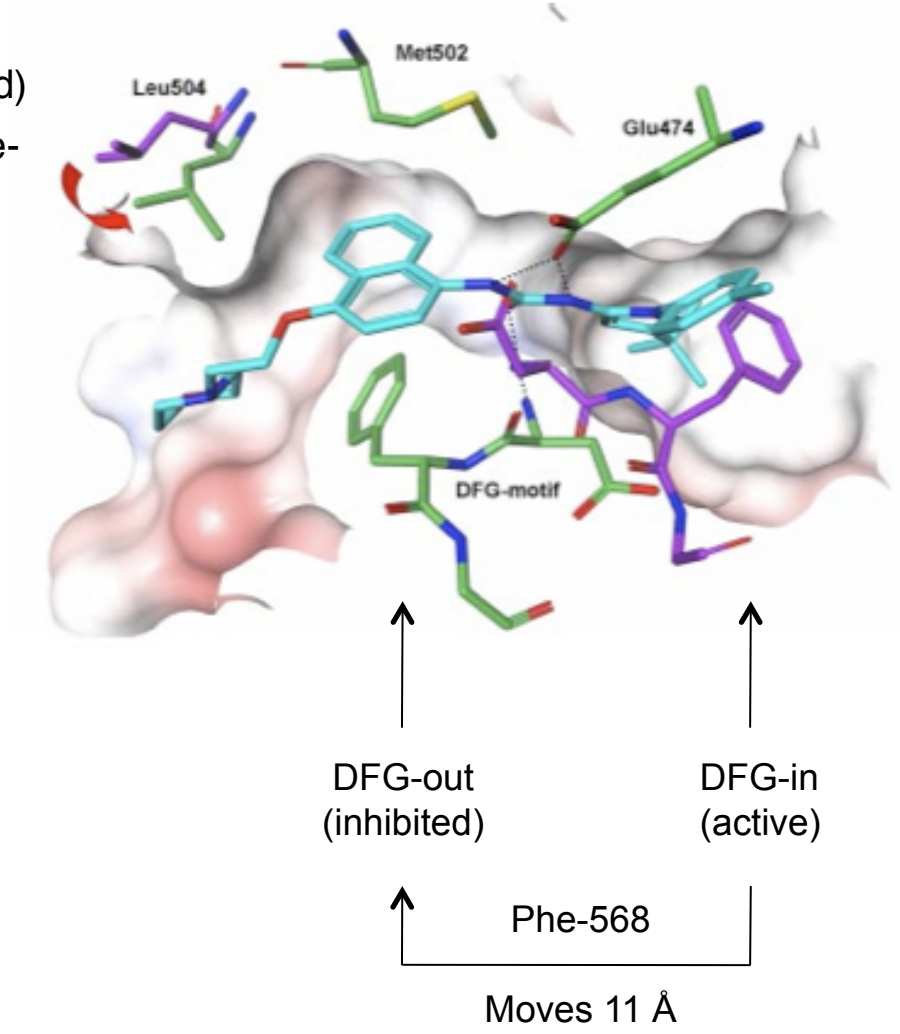
Druker B. J.; *et al. Nature Medicine* **1996**, *2*, 561.

# *Explanation of Gleevec (Imatinib) Selectivity for Bcr-Able Fusion Kinase*

- Imatinib displays "bipartite" binding to both the ATP pocket (conserved) and DFG-loop (not conserved)
- Imatinib binding to Abl causes a unique shift of Phe-568 by 11 Angstroms to block activity
- A similar shift occurs for the DFG loop in PYK2 binding of BIRB796, but the dissimilarity of these loops is telling of the lack of generality of exploitation for kinase inhibitor design



DFG = Aspartic Acid–Phenyl Alanine–Glycine




Han, S.; *et al. J. Bio. Chem.* **2009**, *284*, 13193.

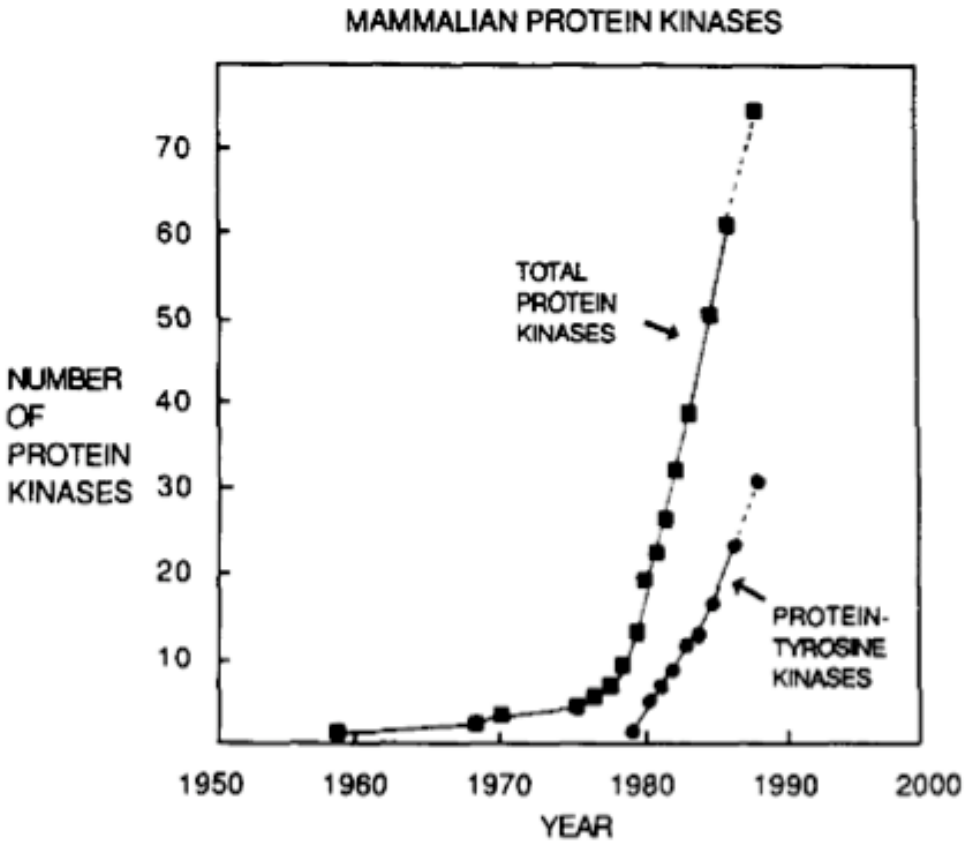
# *High Homology of Protein Kinases: a Curse in Disguise*

- High degree of homology has meant a rapid rate of discovery from molecular cloning of kinase genes

- Appears that all eukaryotic protein Ser/Thr and Tyrkinases evolved from the same gene based on sequence

- For example: cAMP dependent protein kinase shares300 amino acids with pp60 (v-Src) in catalytic domain alone

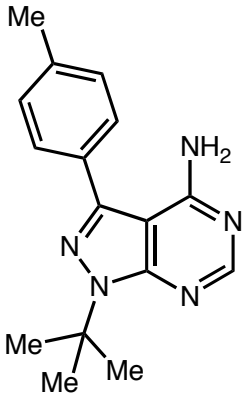
- Downside is that pharmacology alone has trouble finding selective small molecule inhibitors




Hunter T. *Cell* **1987**, *50*, 823.

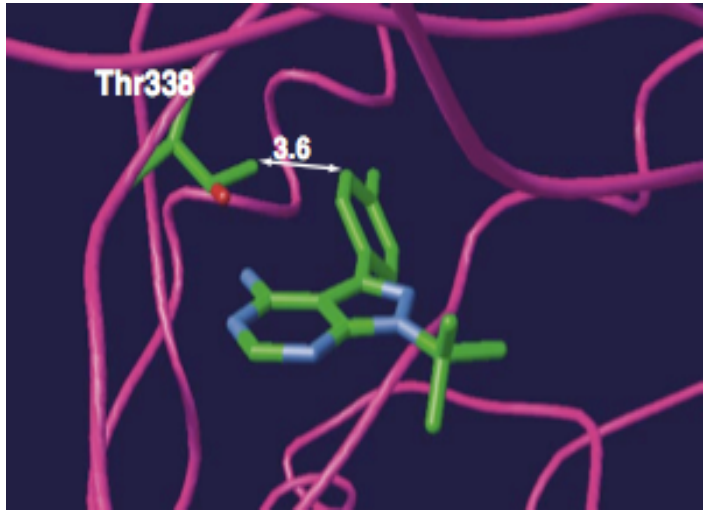
# *PP1 is a Breakthrough in Src-Family Specificity*

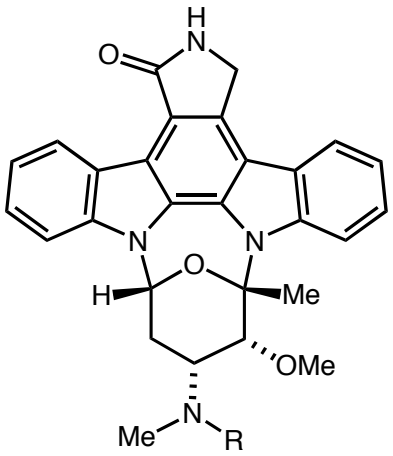
- Hanke discovers PP1 series as selective kinase inhibitors in 1996 which is a more selective class for Src family kinases than previously reported staurospirine.
- Both small molecules are well defined in their role in kinase inhibition; cocrystal structures obtained.




**PP1**, Hanke, 1996

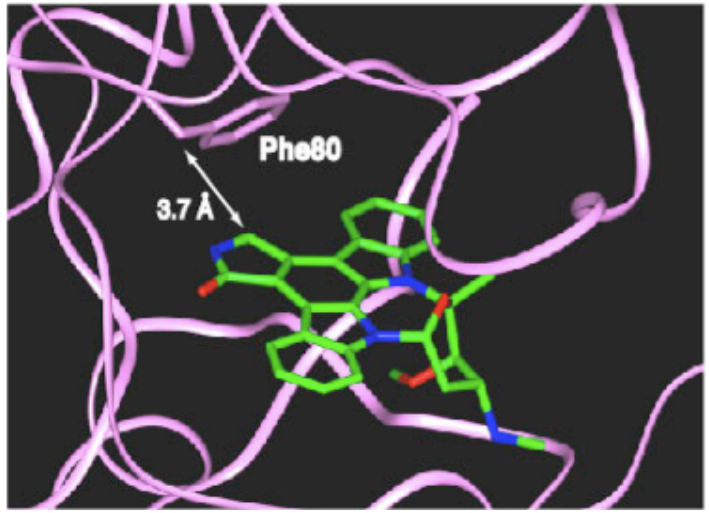
Hck tyrosine kinase



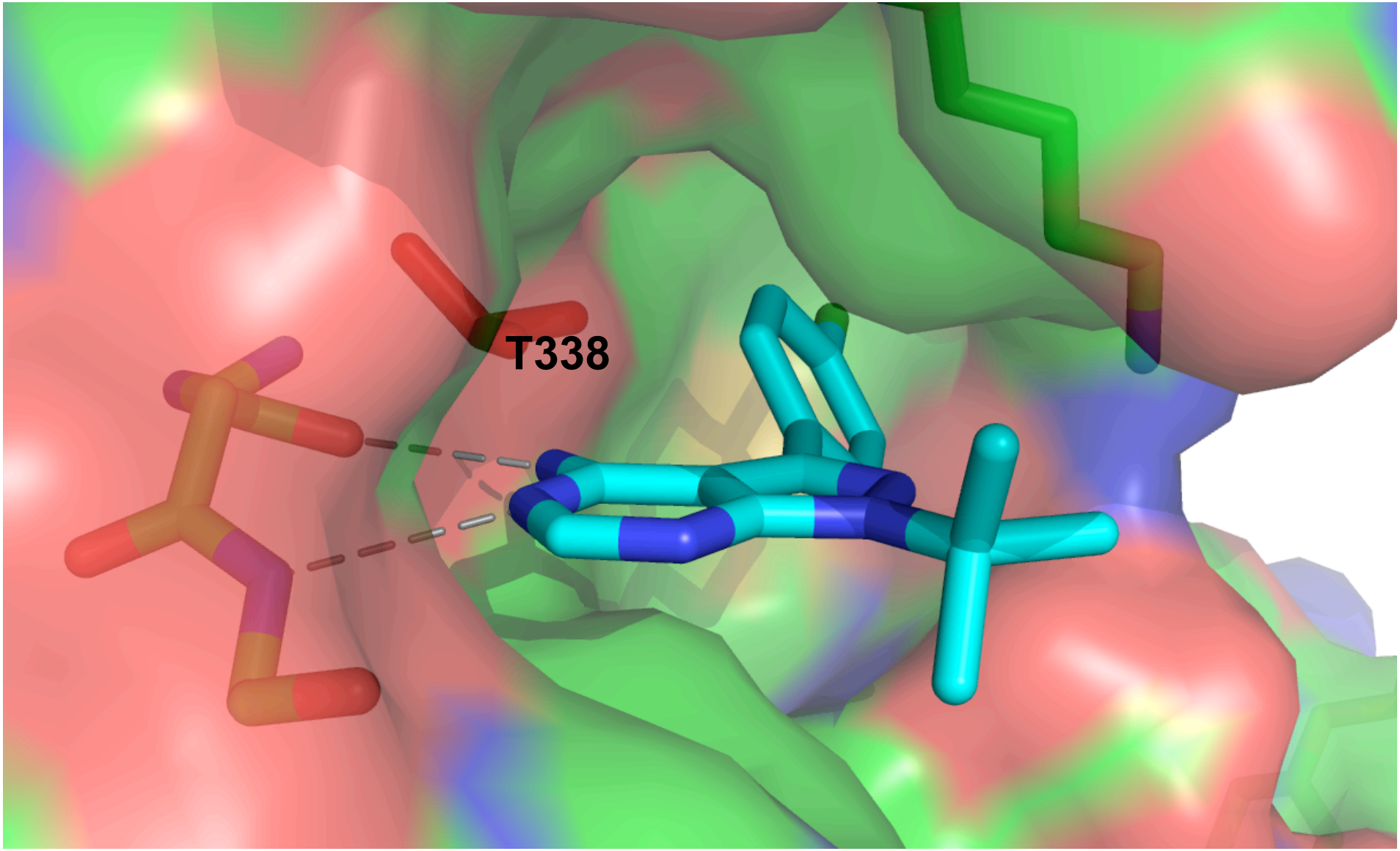

staurosporine (R = H)

CDK2 cell cycle kinase




Hanke, J. H. *et al. J. Biol. Chem.* **1996**, *271*, 695. Shokat, K. M. *et. al. Nature* **2000**, *407*, 395.

*Hck – PP2 Complex with Gatekeeper: T338*



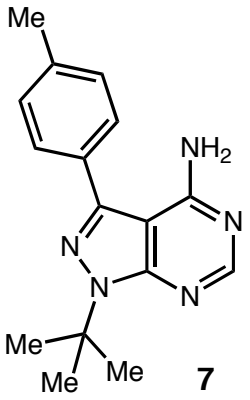

Schindler T., *et al Mol.Cell* **1999**, *3,* 639.

## *New PP1 Analogues Display Unprecedented Binding Affinity and Specificity*

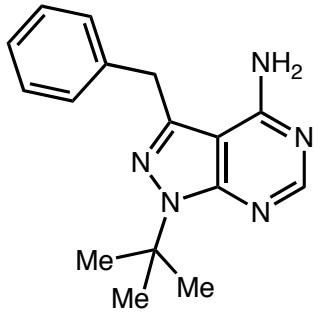
- Analysis of cocrystal structure seggested extension of C7 substituent to increase selectivity
- Inhibitor **9** showed unprecedented activity toward kinase mutants relative to wild type (proof of princeiple)

| | 7 | 8 | 9 |
|---|---|---|---|
| v-Src | 2.2 | 1.0 | 28 |
| c-Fyn | 0.050 | 0.60 | 1.0 |
| c-Abl | 0.30 | 0.60 | 3.4 |
| CDK2 | 22 | 18 | 29 |
| CAMK II | 17 | 22 | 24 |
| v-Src-as1 | | 0.0015 | 0.0043 |
| c-Fyn-as1 | | 0.0065 | 0.0032 |
| c-Abl-as2 | | 0.0070 | 0.12 |
| CDK2-as1 | | 0.015 | 0.0050 |
| CAMKII-as1 | | 0.097 | 0.0080 |

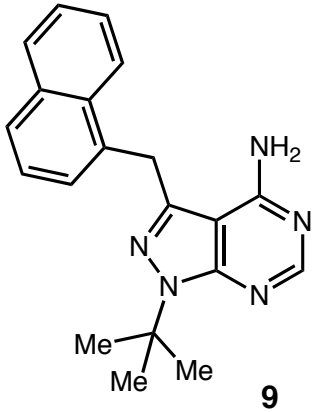
Micromolar $IC_{50}$




7




8



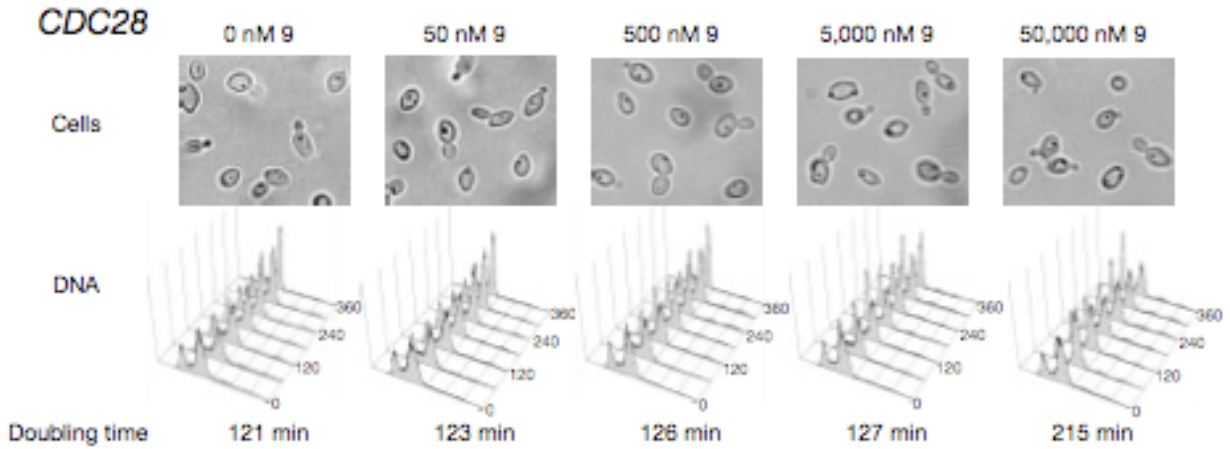

9

Shokat, K. M. *et. al. Nature* **2000**, *407*, 395.

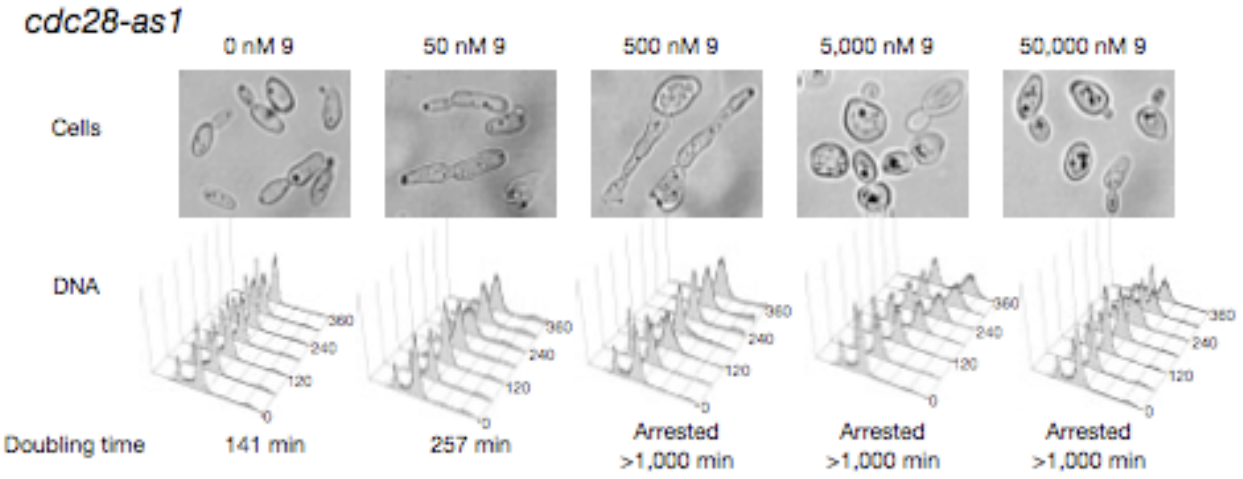
# *PP1 Analogues Show Cell Cycle Arrest of* Cdc28-121 *mutant*

- Cell cycle doubling in budding yeast affectively shut down while wild type cells are unaffected.

Wild Type:



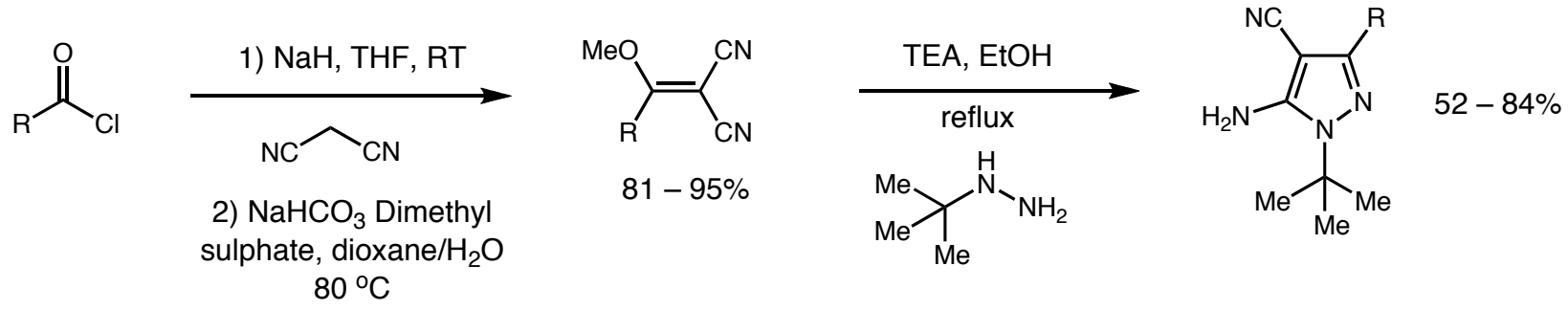

Mutant:

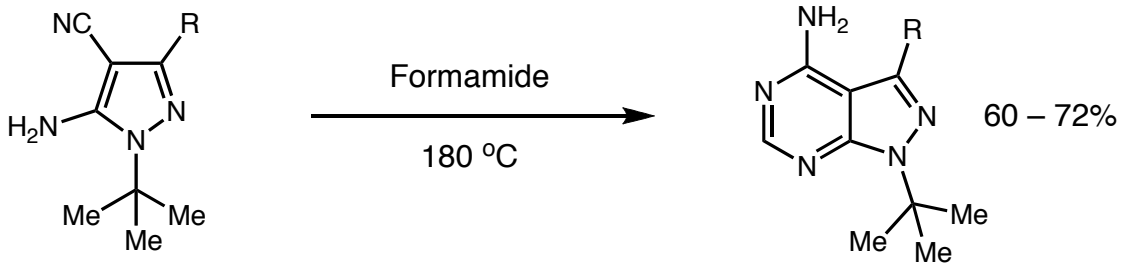



in cell

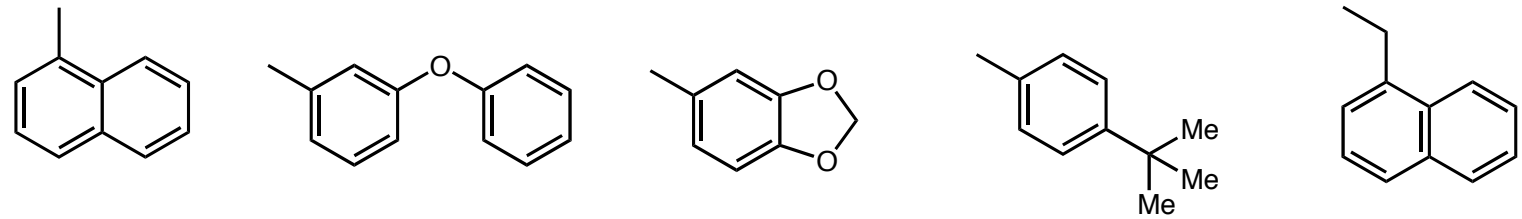
Shokat, K. M. *et. al. Nature* **2000**, *407*, 395.

# *PP1 Inhibitor Synthesis*




R =



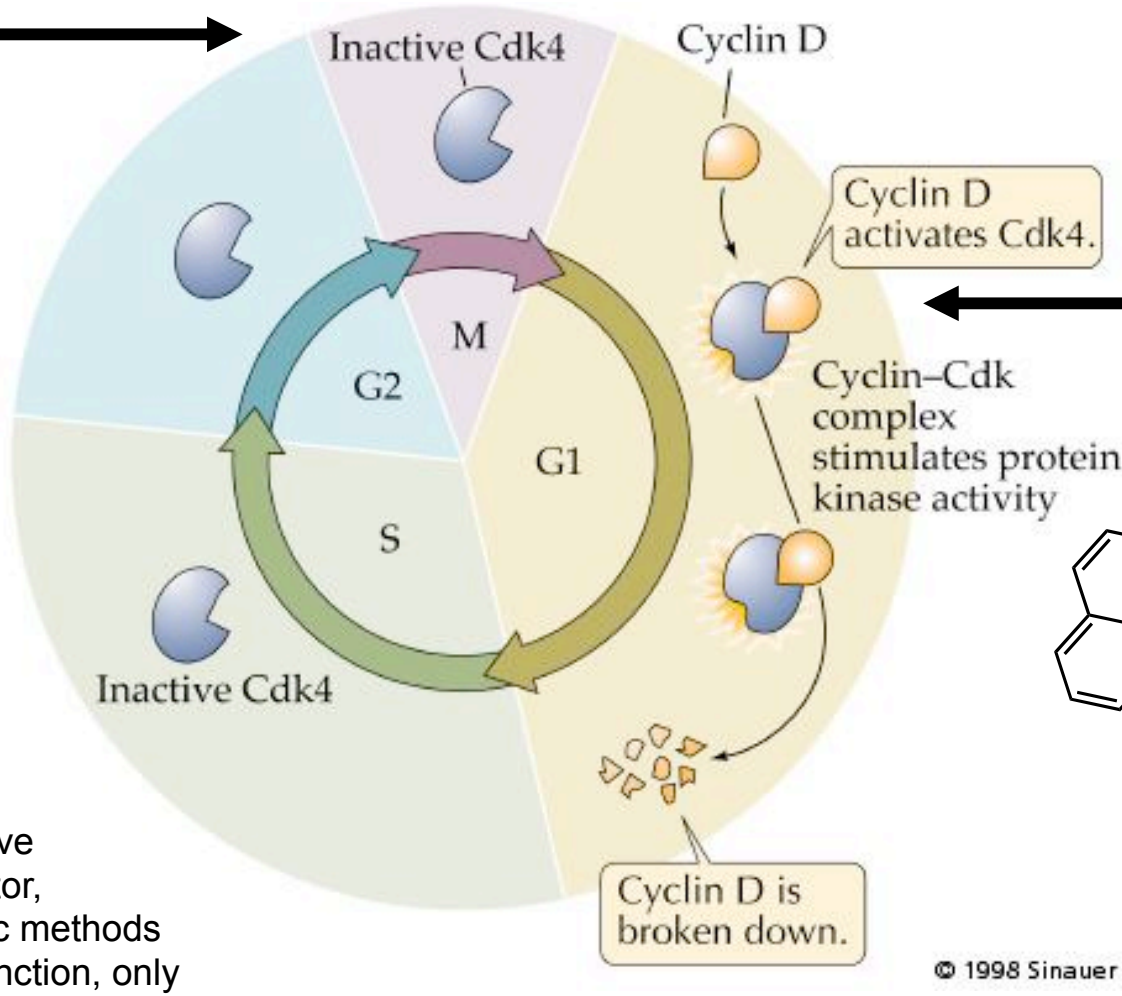
*Examples of Benefits of Chemical Genetics Techniques*

## *Gradational Response Discovered for Cell Cycle Inhibition with ASKA*

- Cell cycle progression assumed to be turned on by CDK2: by knockout of cdc28 or temp. shock
- Bishop and Shultz found a gradational response when PP1-derivative given to analog sensitive cdc28

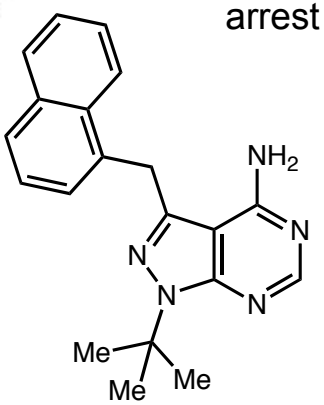
CDK2 →

G1 dependent on high levels of CDK2 expression: low-dose pharmacological arrest




← CDK2

Temperature-sensitive or high-dose pharmacological arrest



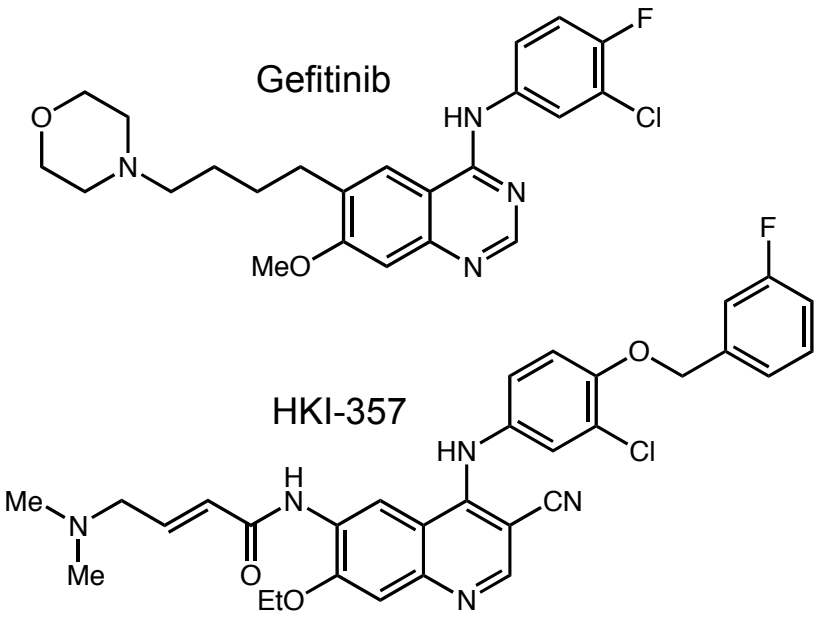

Since wild type kinase is insensitive to chemical inhibitor, and purely genetic methods destroy protein function, only a *chemical genetic* approach reveals the graded response.

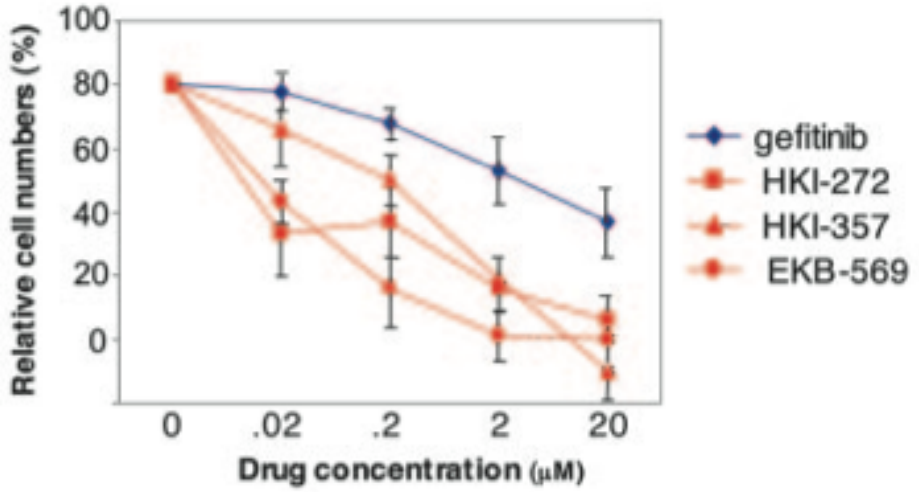

Stern, B.; Nurse, P. *Trends Genet.* **1996**, *12*, 345;
Bishop, D. P.; *et al. Nature* **2000**, *407*, 395.

## *Importance of Gradational Responce in Clinical use of Gefitinib*

- Gefitinib is an inhibitor of the tyrosine kinase domain of epidermal growth factor receptor (EGFR)
- EGFR misregulation implicated in several cancers inlcuding breast and non-small cell lung cancer
- Many patients have tumors resistent to gefitinib; tumors continue to grow
- Resistance is not due to EGFR mutations (as we would expect); tumor lines selected for resistance do not acquire mutations.

- These tumors simply afford resistance by increasing their threshold for EGFR inhibition as a result.
- Since the mode of action is technically still the same, suicide inhibitors are then effective at inhibition.



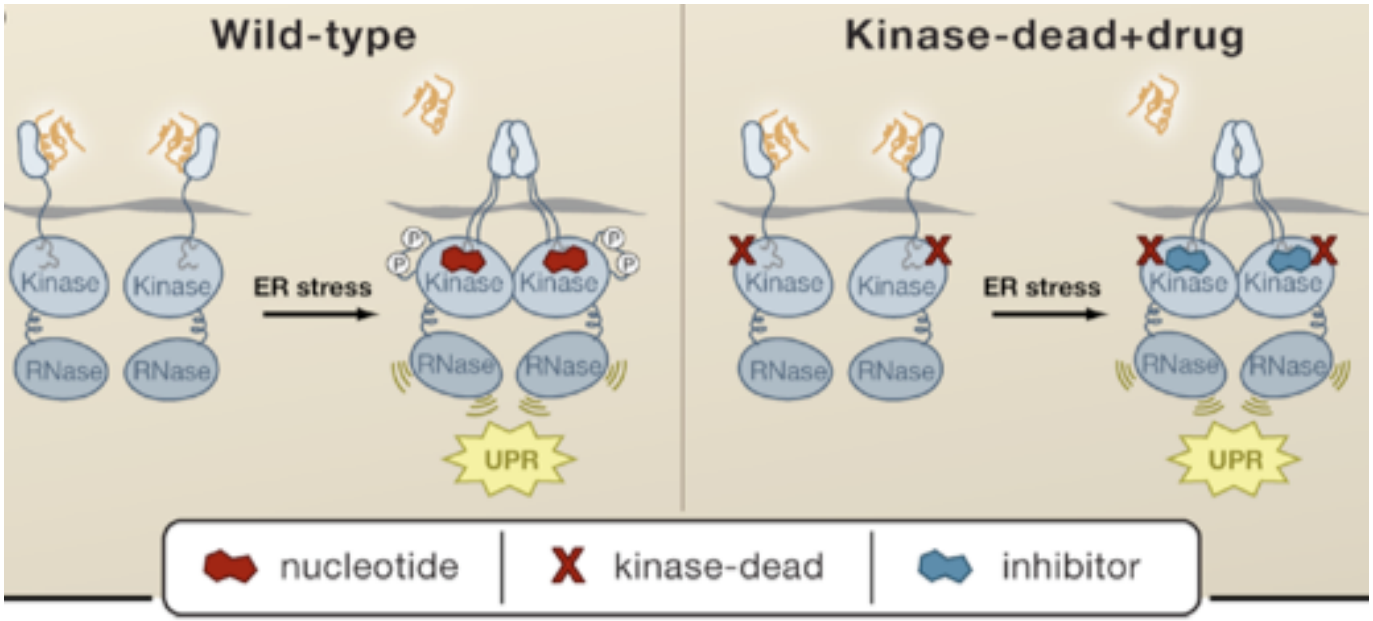




Kwak, E. L.; *et al. Proc. Natl. Acad. Sci. USA* **2005**, *102*, 7665.

*PP1 Uncovers a Mode of Action for the Unfolded Protein Response*

- Knockout of Ire1 or expression of a kinase-dead allele blocks the unfolded protein response (UPR)
- An ATP competitive inhibitor of the kinase-dead allele rescues the UPR
- This means that $PP_1$ acts as an $Ire_1$ agonist rather than an $Ire_1$ inhibitor, even though it binds to the $Ire_1$ active site and directly blocks kinase activity (!).

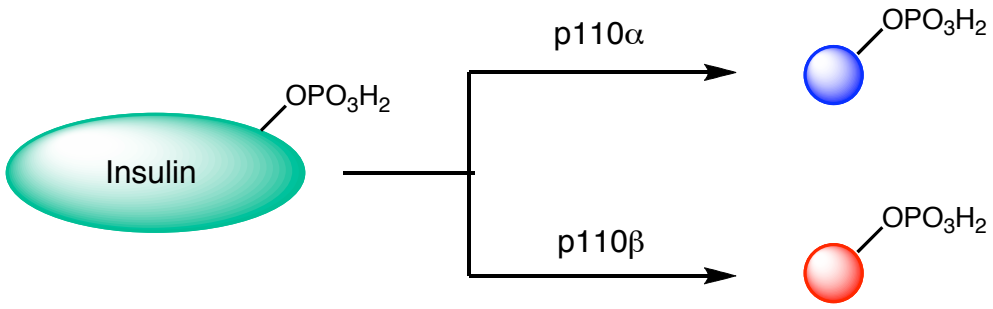
- Explanation: An ATP competitive ligand for the Ire1 kinase domain allosterically activates the Ire1 RNAse domain during the UPR




Patil, C., Walter, P. *Curr. Opin. Cell Biol.* **2001**, *13*, 349;
Papa, F. R., Zhang, C., Shokat, K. M.; Walter, P. *Science* **2003**, *302*, 1533.

## *Importance of Relative Enzyme Stoichiometry Masked by Knockout*

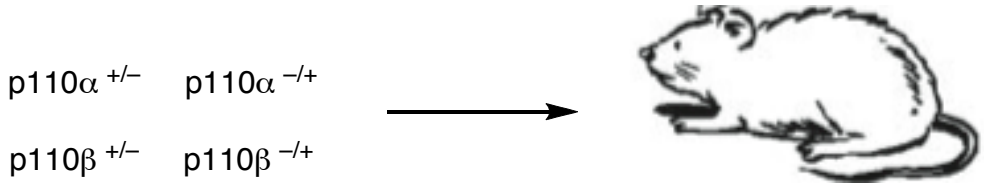
- Both PI3 kinases, p110α and p110β, carry signals from the growth factor, insulin




Bi, L.; *et al. Mamm. Genome* **2002**, *13*, 169.

## *Importance of Relative Enzyme Stoichiometry Masked by Knockout*

- Both PI3 kinases, p110α and p110β, carry signals from the growth factor, insulin
- Because knockout of either kinase isoform kills mice early in development we know that they cannot compensate for each other but don't know their roles
- Heterozygous deletion of either produces no phenotype; deletion of p85, the p110 binding partner, paradoxically increases insulin signaling




Same as WT

Bi, L.; *et al. Mamm. Genome* **2002**, *13*, 169.

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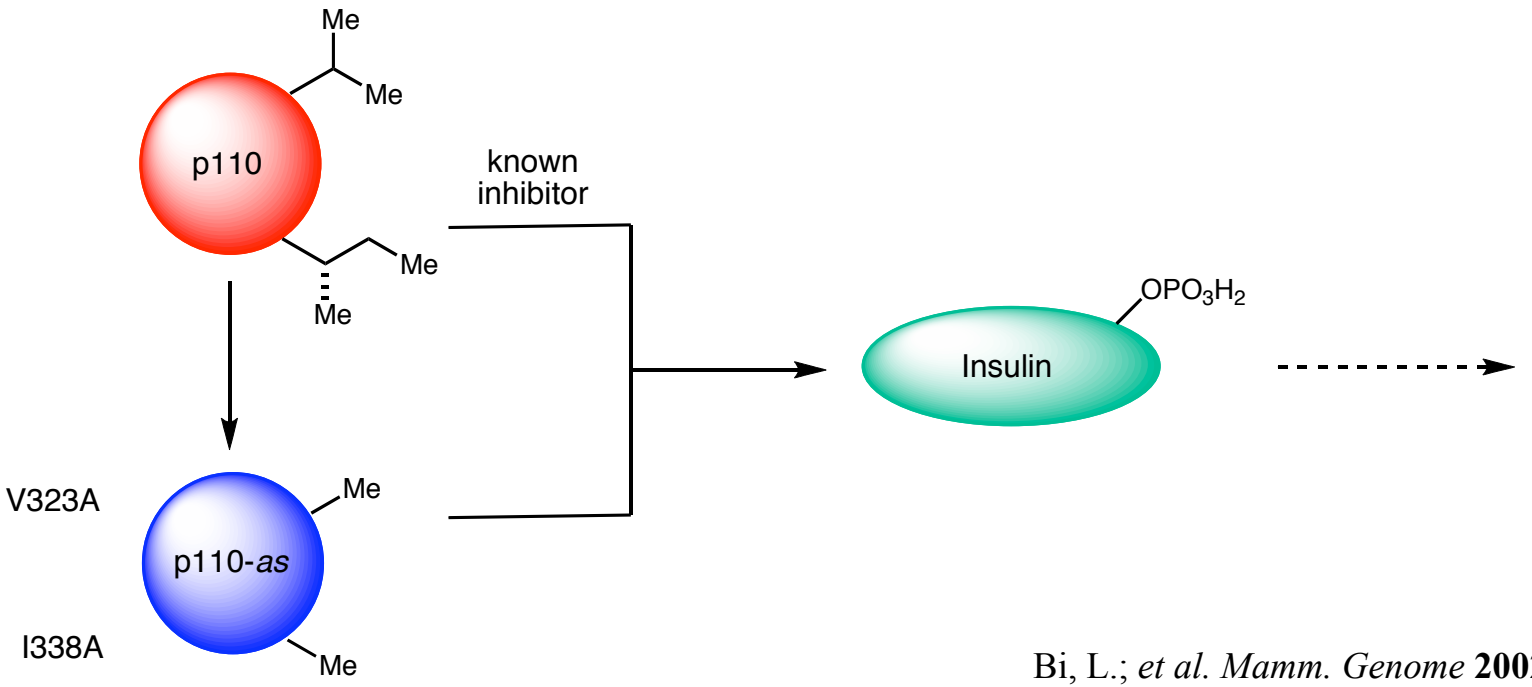


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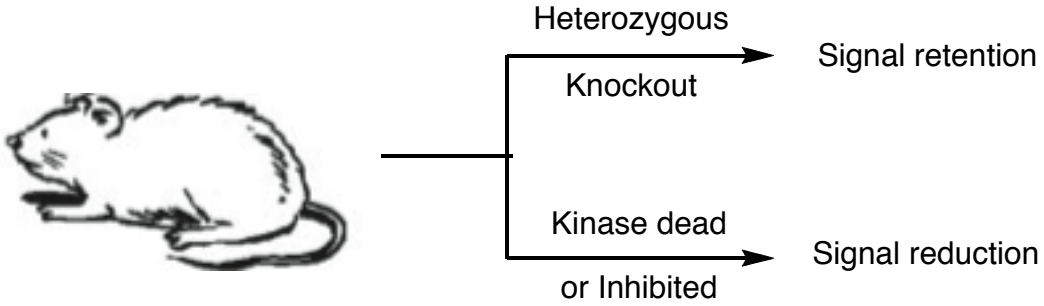
- Paradox: why do mice lacking p110 or p85 show normal or increased insulin signaling while kinase-dead mutants (or their equivalent p110$\alpha$-inhibited mice) show a reduction?

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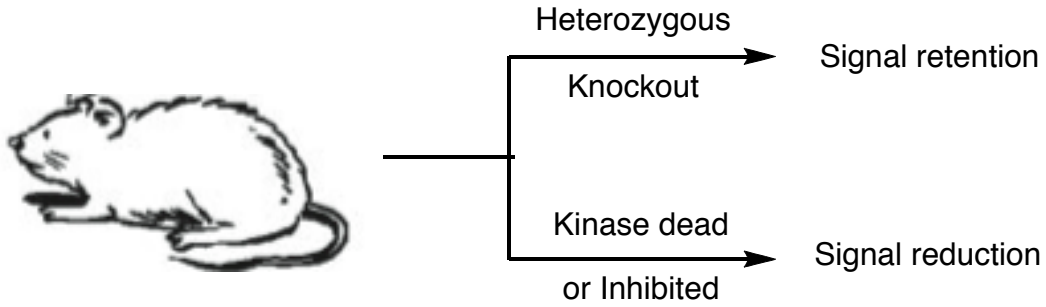
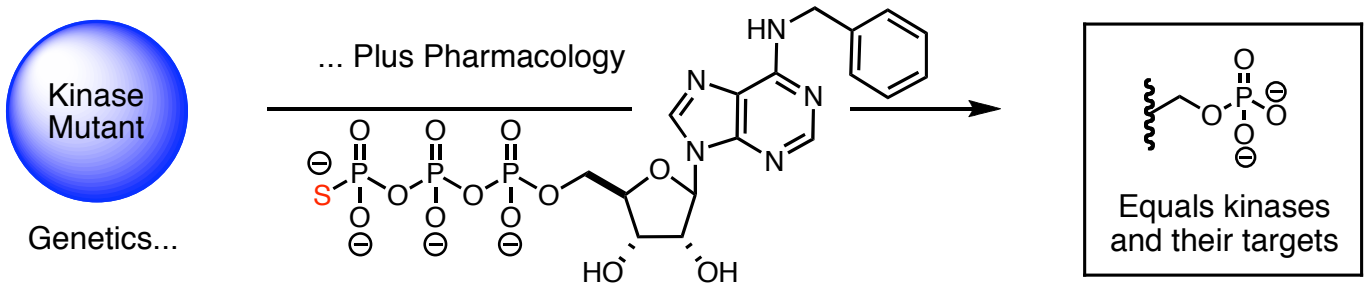
- Both knockin kinase-dead mice as well as mice treated with a p110α–selective inhibitor show reduced insulin signaling
- Paradox: why do mice lacking p110 or p85 show normal or increased insulin signaling while kinase-dead mutants (or their equivalent p110α-inhibited mice) show a reduction?
- Resulting Model: since p85 can function as a negative regulator of p110, insulin transduction controlled by relative stoichiometry of p110 to p85 rather than absolute amounts.




Bi, L.; *et al. Mamm. Genome* **2002**, *13*, 169.

# *Chemical Genetics Techniques for Substrate Identification*

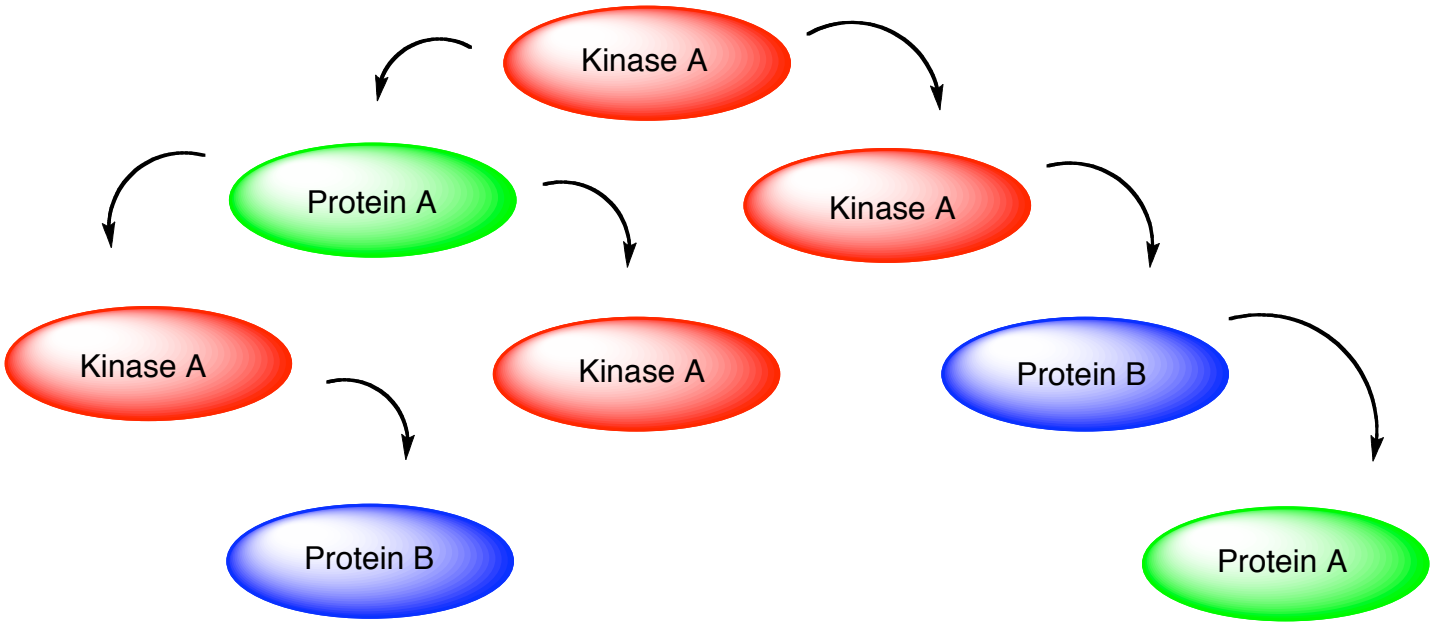


*Previous Phospho-Protein Substrate Identification Methodology*

- **MALDI-TOF mass spectrometry** of cell lysates requires high concentration of desired phospho-protein substrate relative to non-desired phosphate-containing lysates

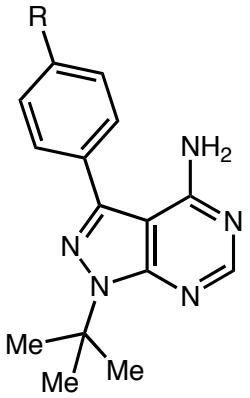
- **Protein lysate chromatography** involves analyzing individual column fractions for their activity against a particular kinase; tedious and has problem that kinases are promiscuous in vitro

- **Yeast two hybrid screen** not applicable when a third party protein is involved



# *PP1 Inhibitor Usage Contrasted with Bumped* N*-Bn ATP Analog Usage*

- PP1 inhibitor derivatives serve to study kinase signaling pathways by inhibition




**PP1**: Known kinase inhibitor

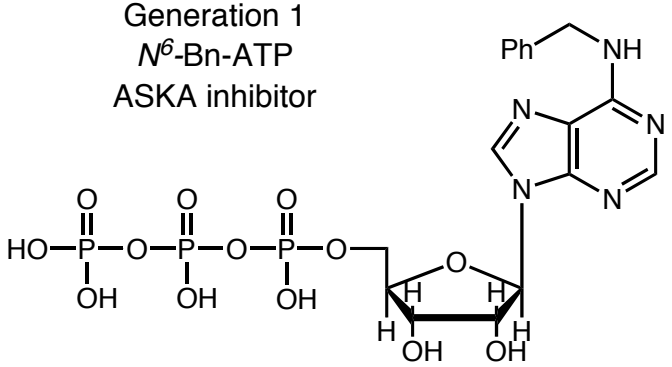
analog sensitive kinase →

Kinase inhibition

No substrate identification

- ATP analogs allow for substrate identification through gamma phosphate manipulation

Generation 1
$N^6$-Bn-ATP
ASKA inhibitor




analog sensitive kinase →

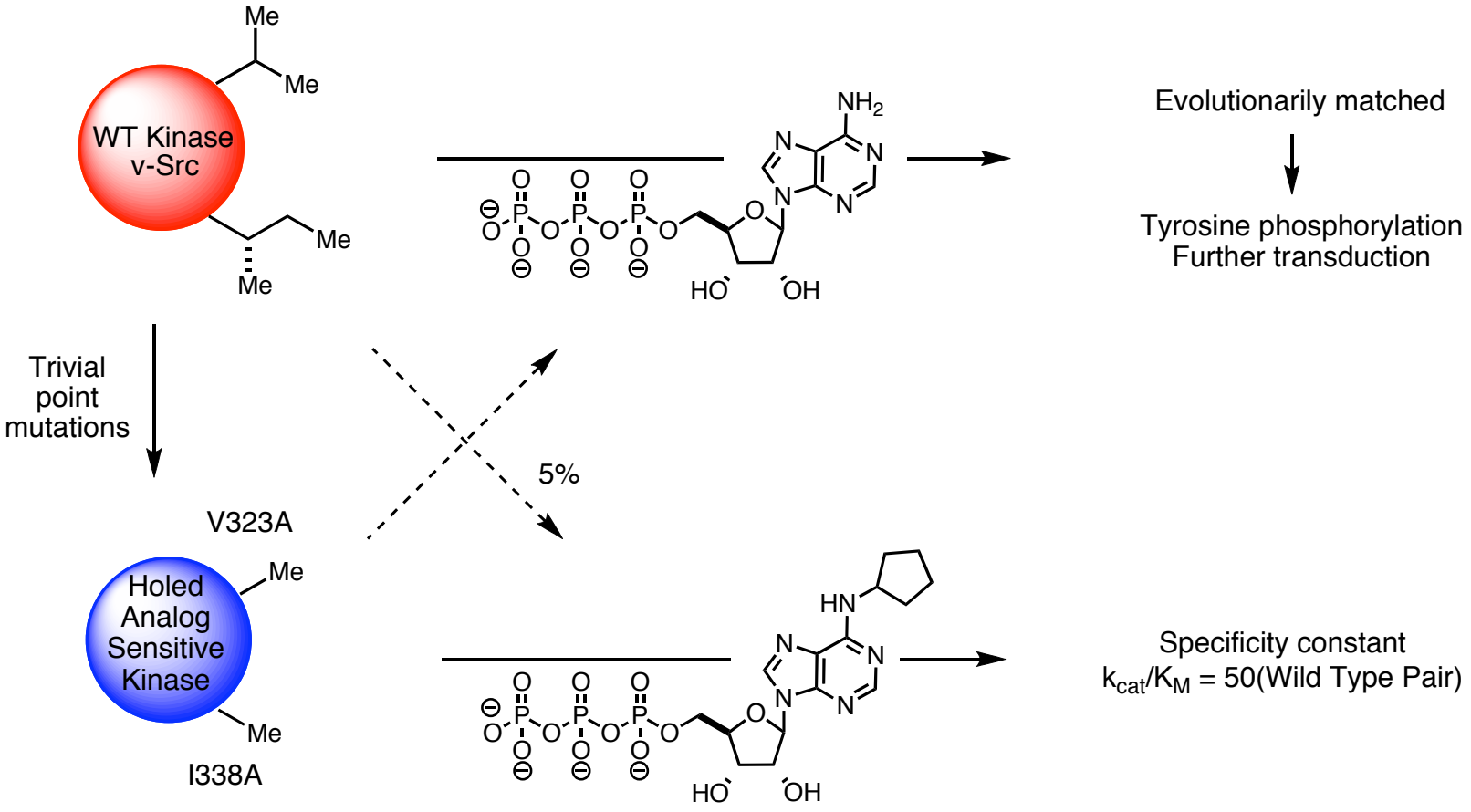
Substrate Identification

via:

$^{32}$P radio labelling or

gamma-thio phosphate

# *First Example of Potent and Selective ATP Analog*




Shokat, K, M,; *et al. Proc. Natl. Acad. Sci. USA* **1997**, *94*, 3565.

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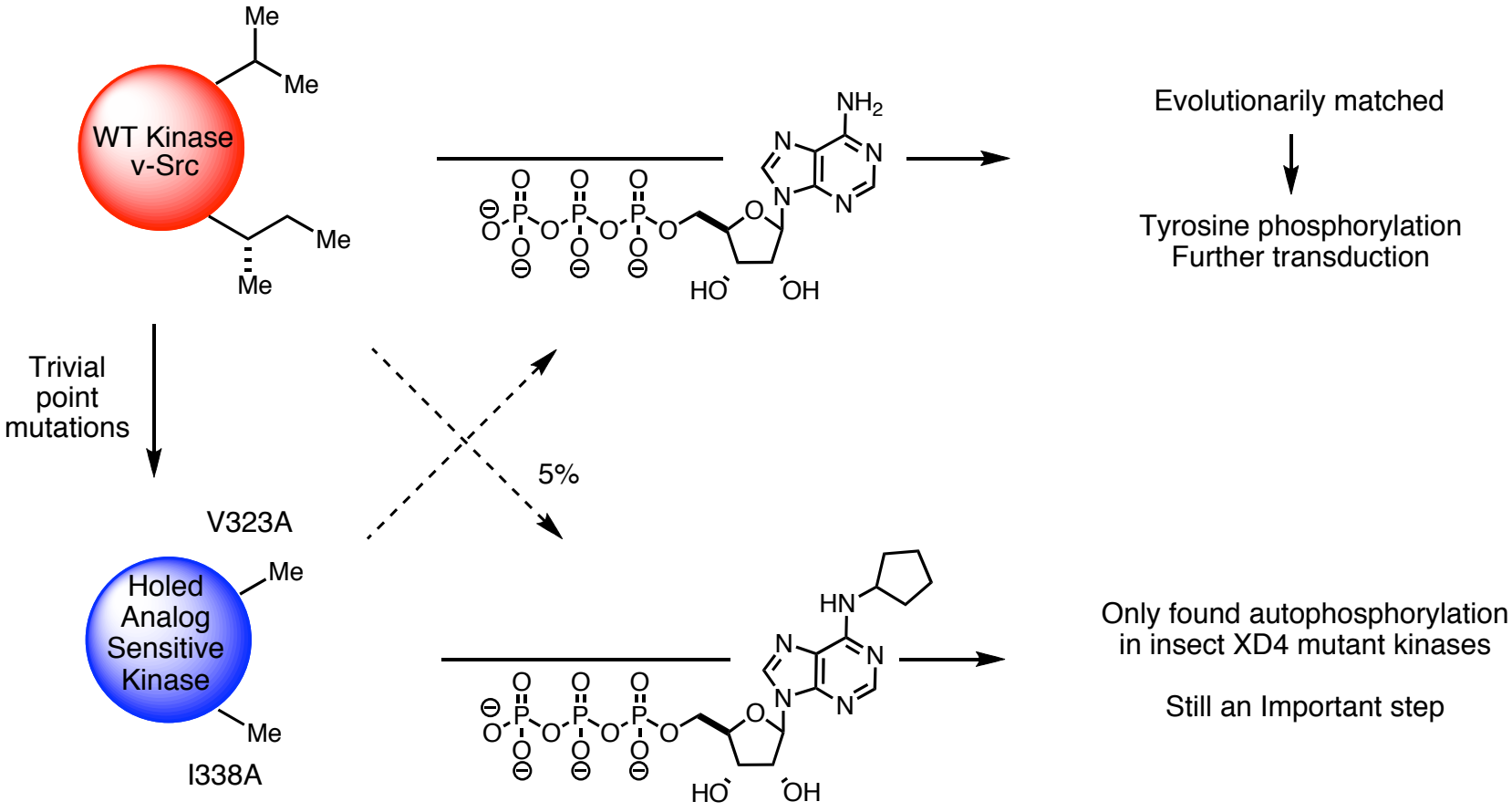


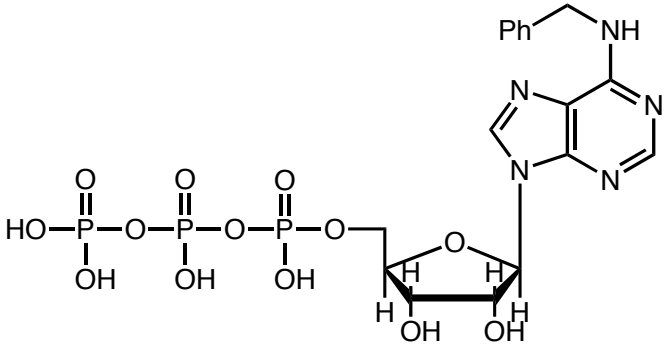

Shokat, K, M,; *et al. Proc. Natl. Acad. Sci. USA* **1997**, *94*, 3565.

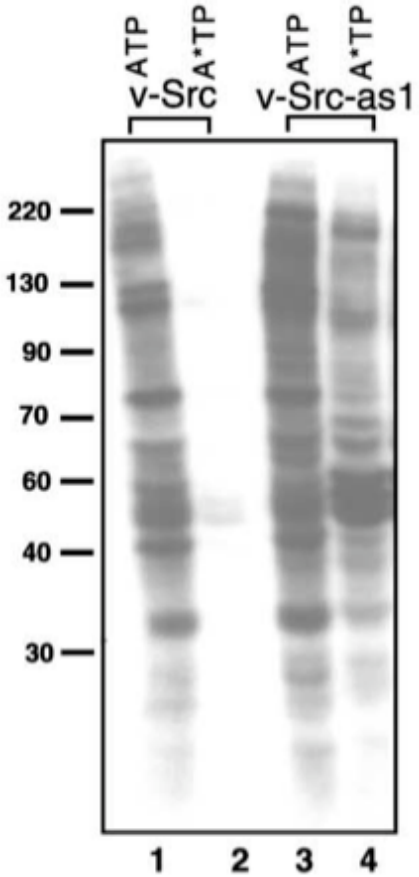
# *ASKA Strategy Leads to Identification of Novel v-Src Targets*

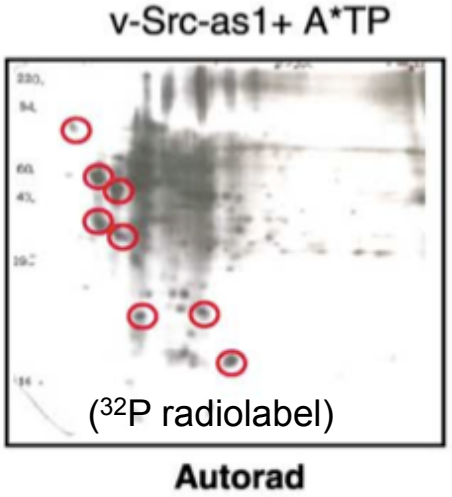
- v-Src phosphorylates tyrosine on 50 proteins but any could come from phosphorylation events from other kinases that v-Src interacts with.

- Radiolabeling of an ATP analog reveals precise phosphotyrosine substrates since the interaction orthogonally adds a $^{32}PO_4^{2-}$ radiolabel only to v-Src-as1 (analog sensitive mutant) targets.

- Cofilin not a known phosphotyrosine product of v-Src; results presumed faulty. ASKA strategy confirms Cofilin and calumenin as novel targets of oncogene v-Src.



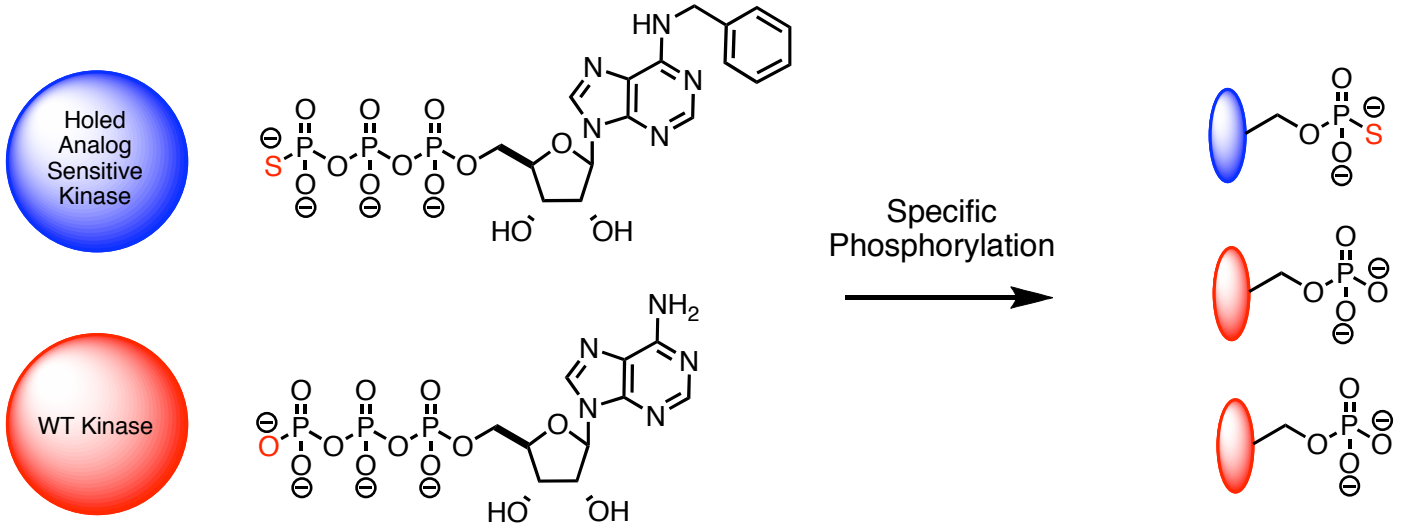




in cell

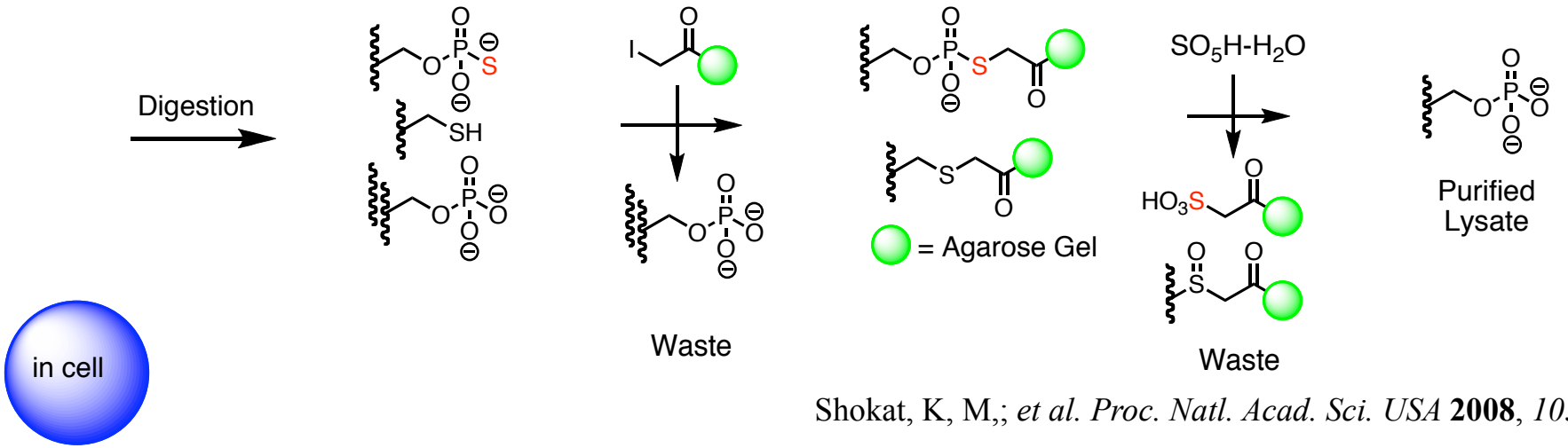
Shah, K.; Shokat, K. M. *Chem. Biol.* **2002**, *9*, 35.

# *Thio-Phosphate Tagging of as-Kinase Direct Phosphorylation Products*

■ Gamma thio *N*-Bn ATP selectively binds to Cdk1-cyclin B (F80G) then yielding chalco-differentiated tyrosine phosphorylation targets



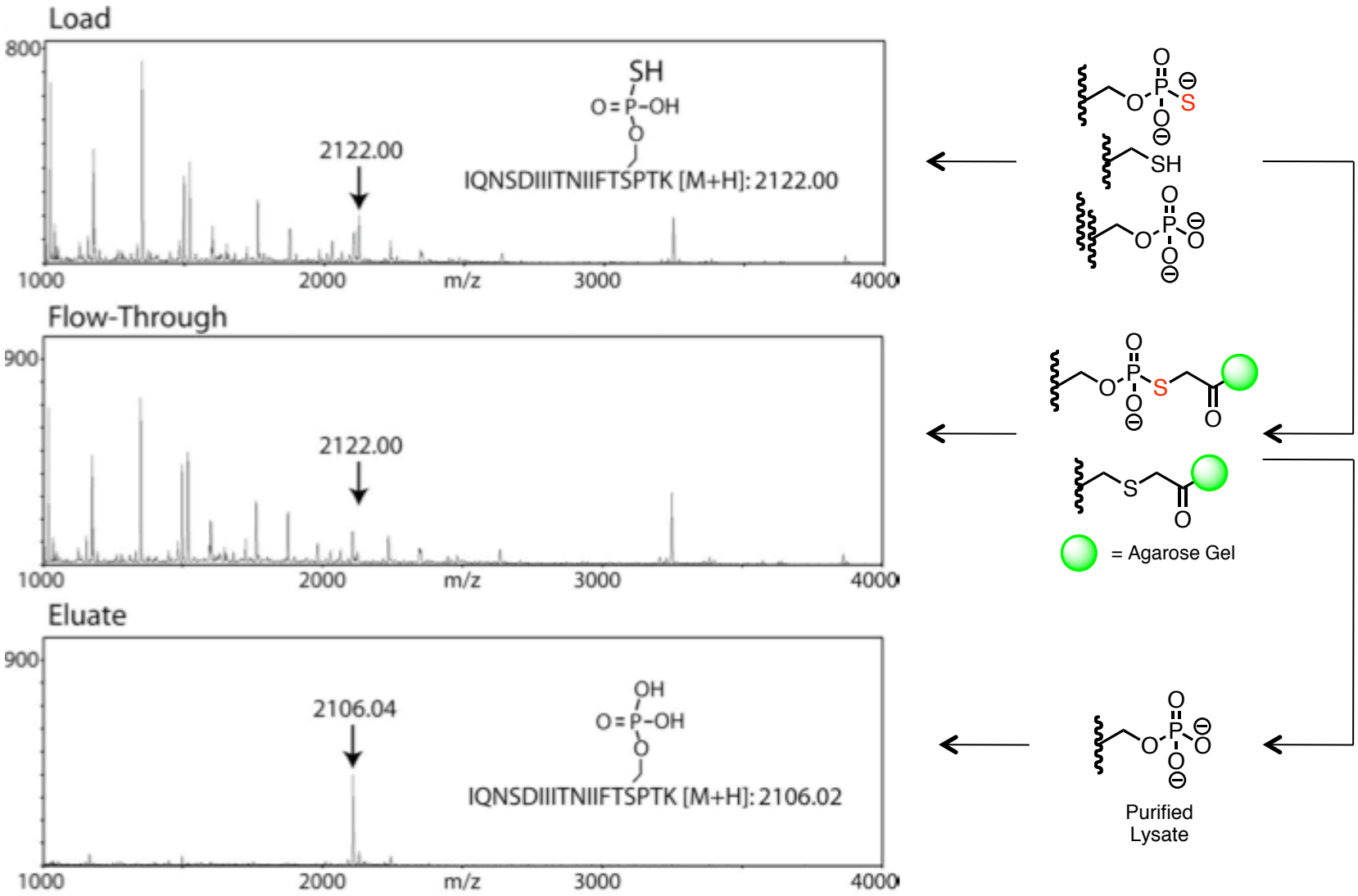

■ After lysis, oxo-phosphate derivatives are removed by selective alkylation; cystein products are washed away via Oxone oxidation, concommitantly re-oxidizing thio-phosphate




Shokat, K, M,; *et al. Proc. Natl. Acad. Sci. USA* **2008**, *105*, 1442.

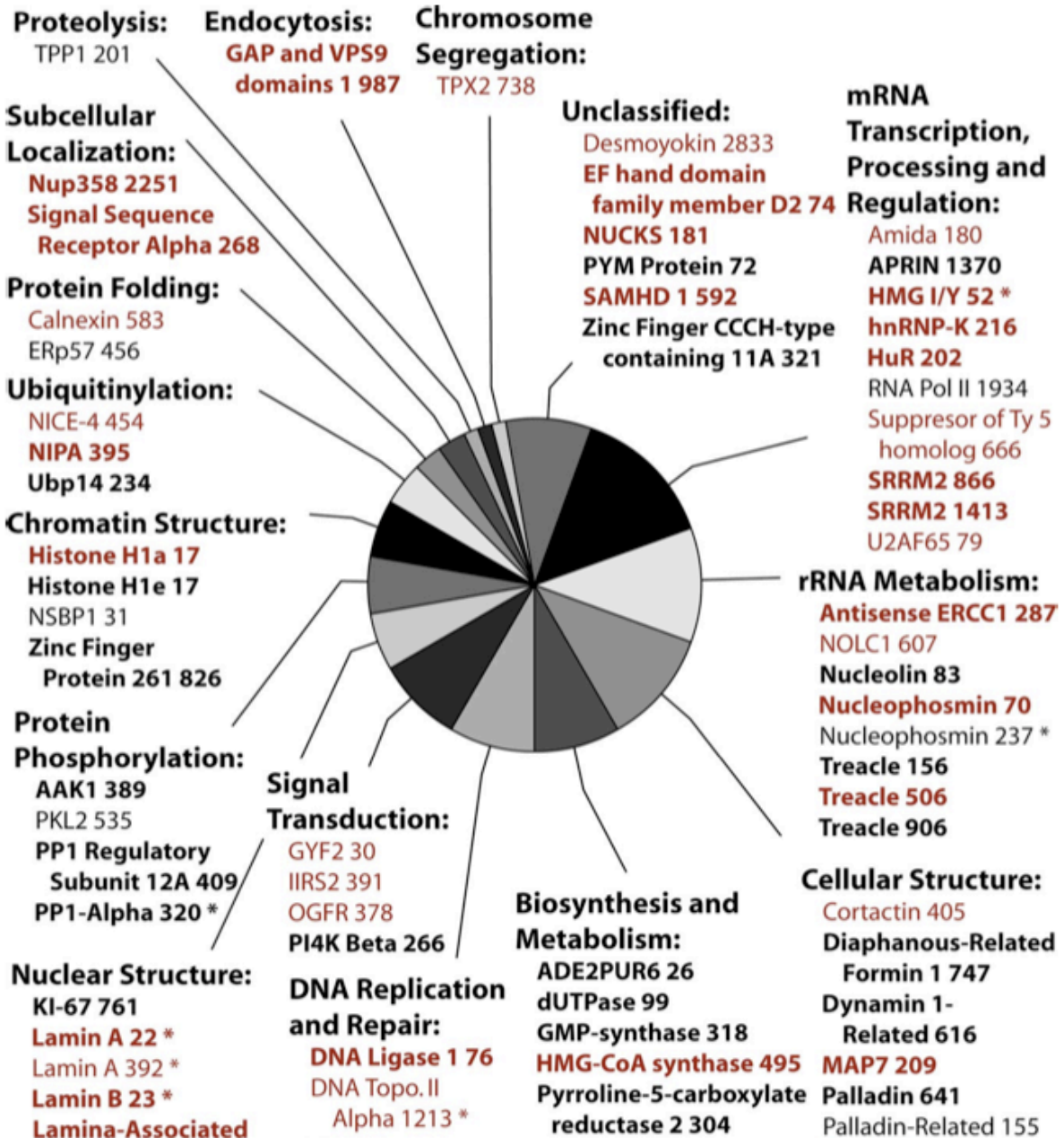
# *Thio-Phosphate Tagging of as-Kinase Direct Phosphorylation Products*

- MALDI-TOF analysis of each stage of substrate purification




Shokat, K, M,; *et al. Proc. Natl. Acad. Sci. USA* **2008**, *105*, 1442.

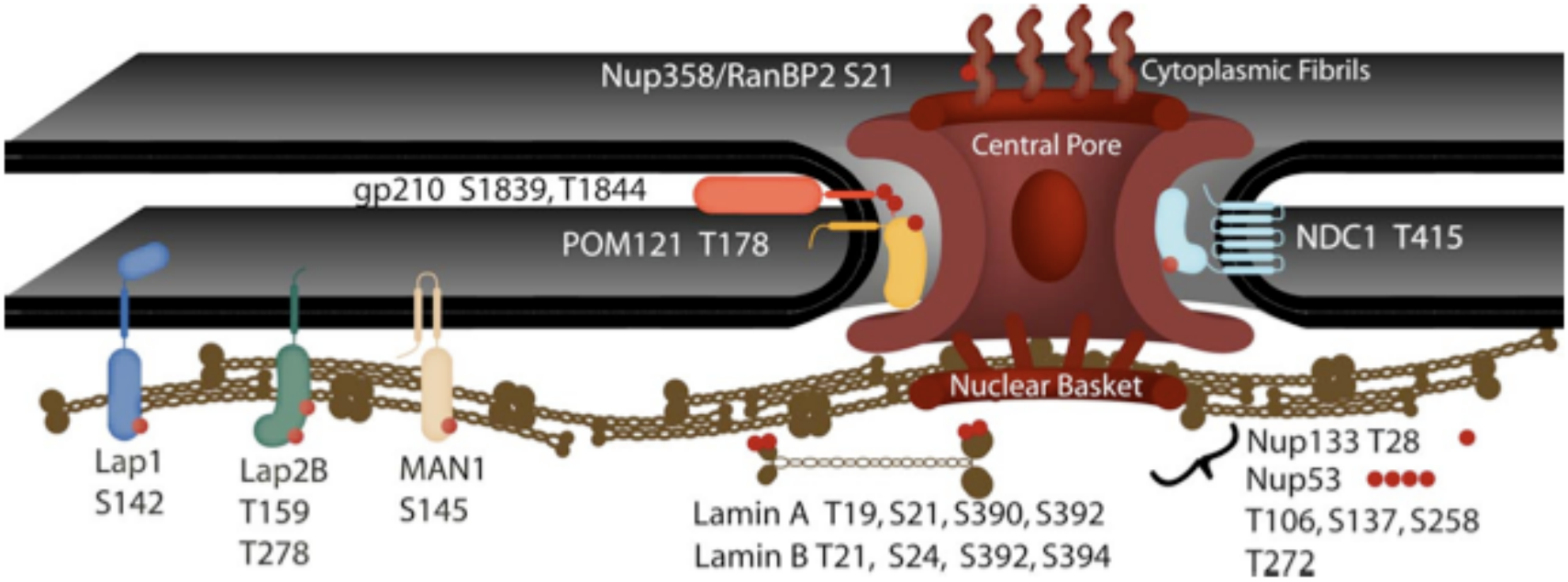
*Thio-Phosphate Tagging of as-Kinase Direct Phosphorylation Products*




in cell

## *Thio-Phosphate Tagging of as-Kinase Direct Phosphorylation Products*

- Protocol next used to study phosphorylation sites on the nuclear pore complex (NPC) and nuclear lamina, both comprising the nuclear envelope (known target of Cdk activity)
- Rediscovered known phosphorylation sites and discovered new ones

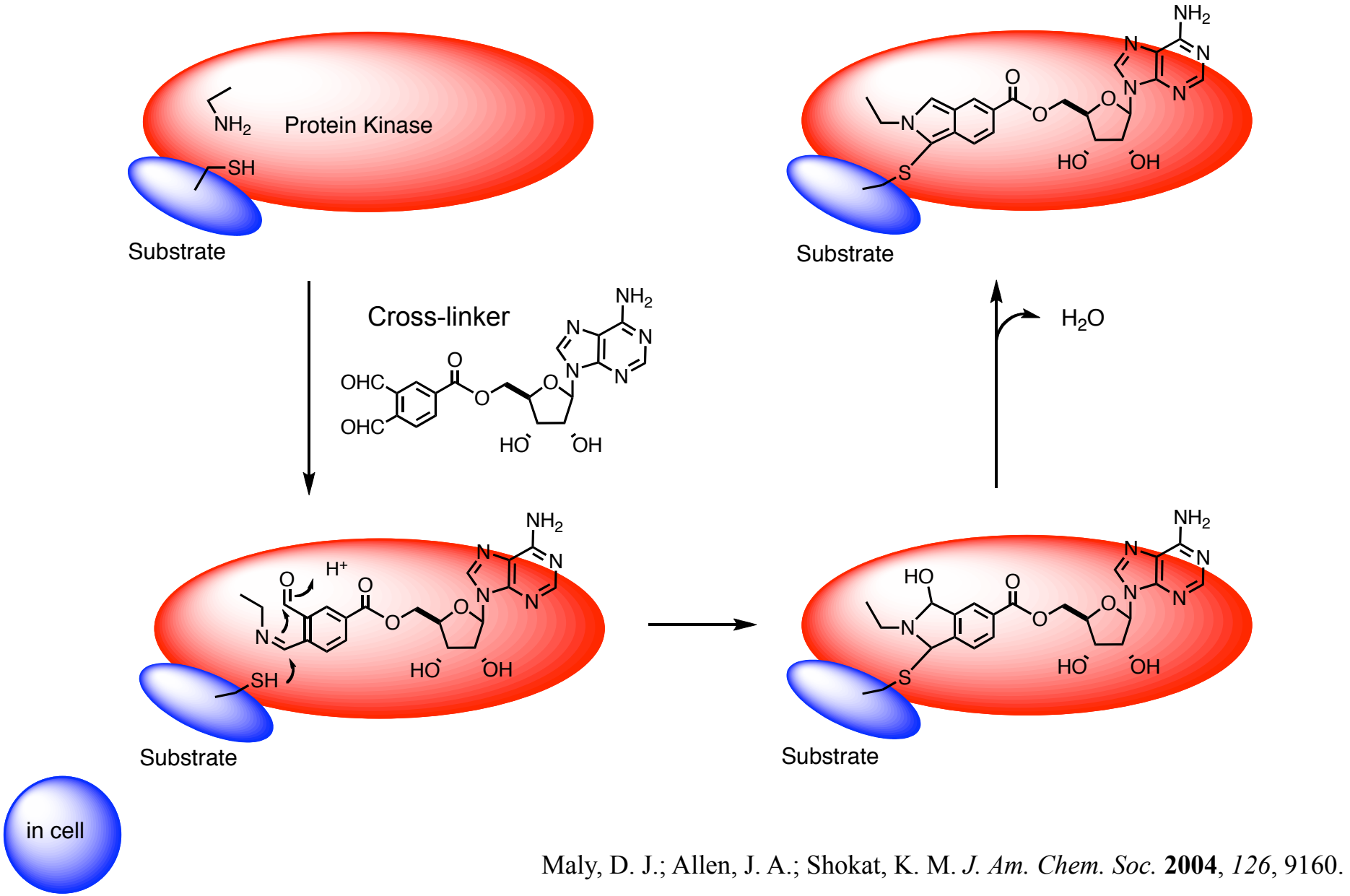



in cell

Shokat, K, M,; *et al. Proc. Natl. Acad. Sci. USA* **2008**, *105*, 1442.

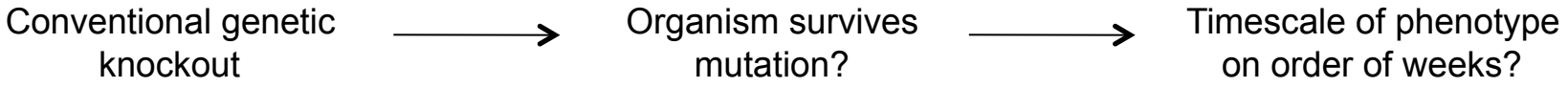
# *Variable Reversal: Hold Substrate Constant*

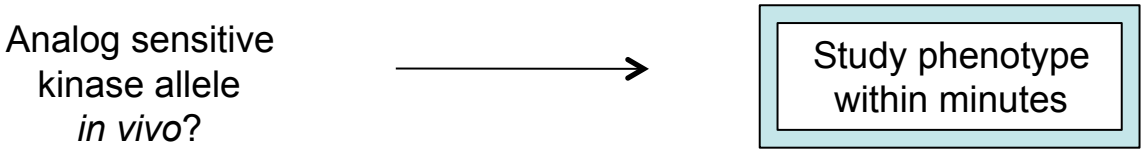
- Mutate substrate of interest (cysteine) and cross-link to discover kinase target




Maly, D. J.; Allen, J. A.; Shokat, K. M. *J. Am. Chem. Soc.* **2004**, *126*, 9160.

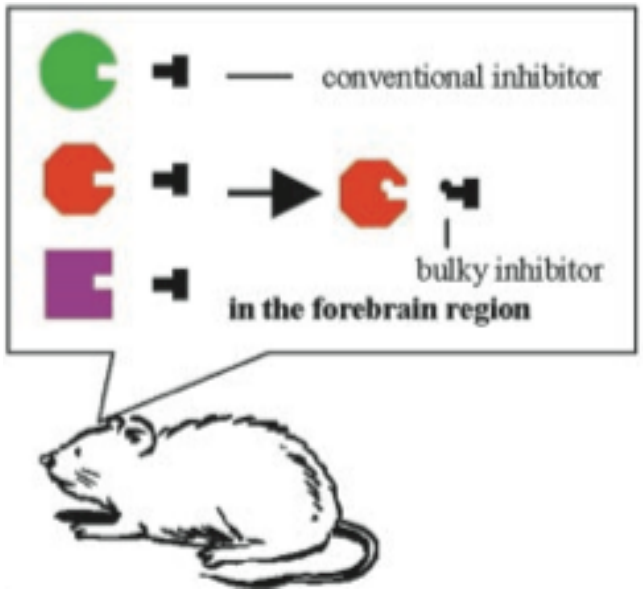
# *True Power of Chemical Genetics: Temporal Control*

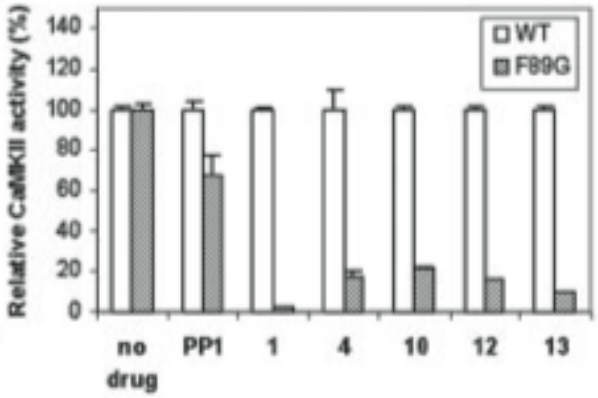


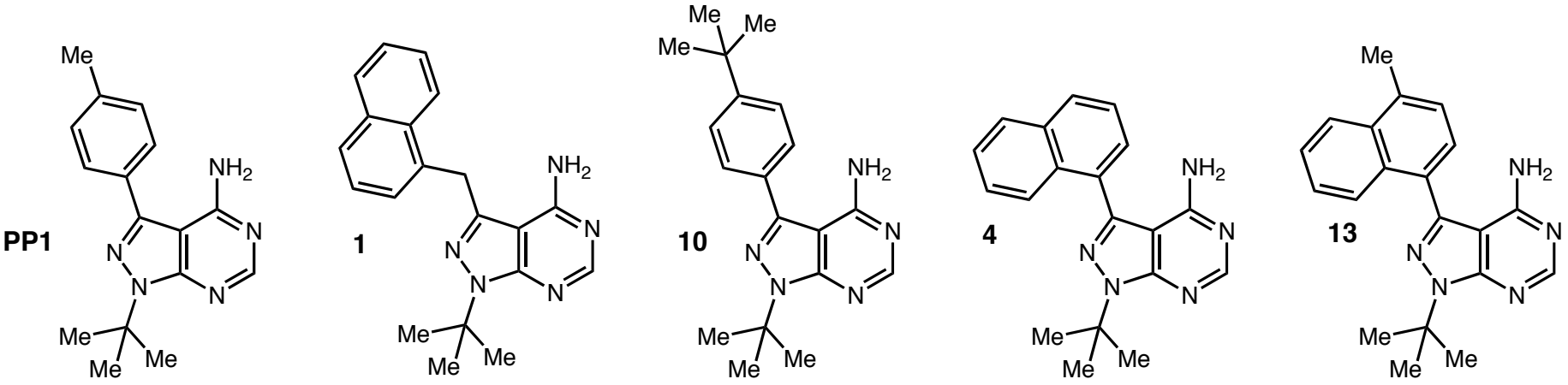



# *True Power of Chemical Genetics: Temporal Control*


- Can we direct the knockout at the protein level rather than at the DNA level?
- PP1 derivative found to be potent for of α-$Ca^{2+}$/calmodulin-dependent protein kinase II (αCAMKII)



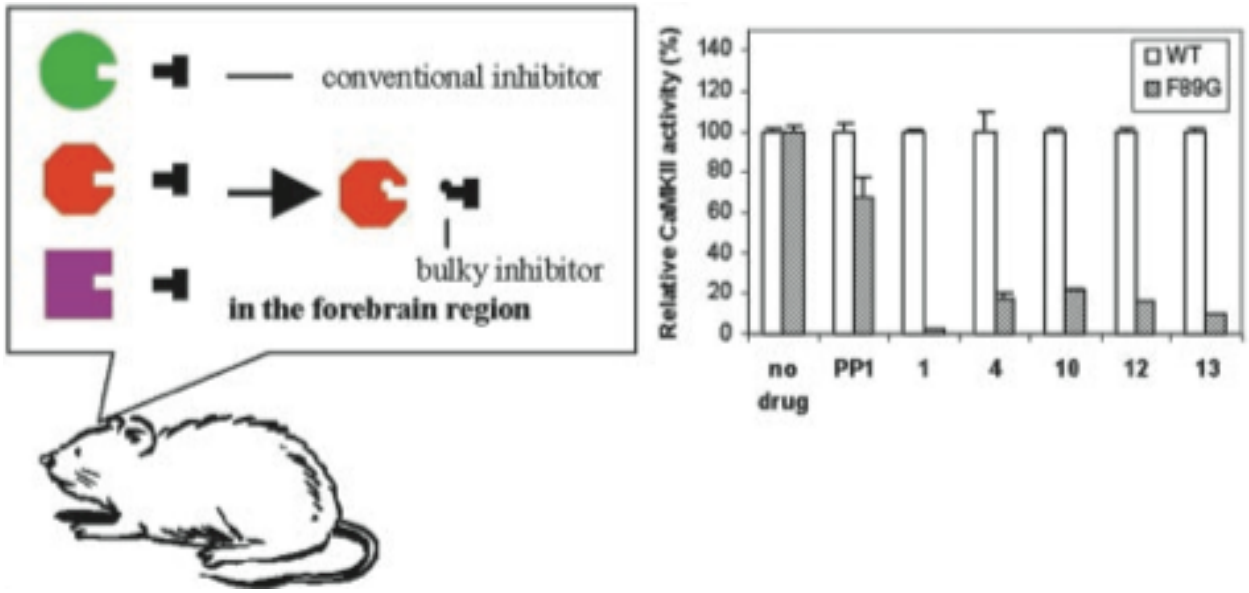




Shokat, K. M.; Tsien, J. Z.; *et al. Proc. Natl. Acad. Sci. USA* **2003**, *100*, 4287.

## *True Power of Chemical Genetics: Temporal Control*

- Can we direct the knockout at the protein level rather than at the DNA level?
- PP1 derivative found to be potent for of $\alpha$-$Ca^{2+}$/calmodulin-dependent protein kinase II ($\alpha$CAMKII)

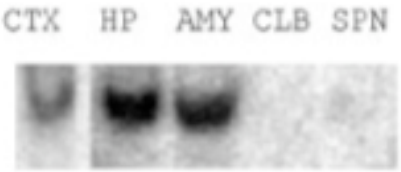



- $\alpha$CAMKII studied in relation to contextual (hippocampal) and fear-based (amygdala-based) conditioning
- Produced mice overexpressing $\alpha$CAMKII-F89G in forebrain, hippocampus and amygdala (mRNA levels)
- Tritium incorporated NM-PP1 (**1**) enters forbrain in 3 - 5 min, peaks at 20 min, bases out at 45 min.

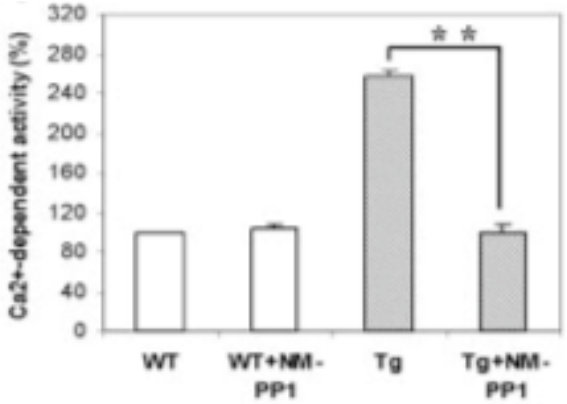
Shokat, K. M.; Tsien, J. Z.; *et al. Proc. Natl. Acad. Sci. USA* **2003**, *100*, 4287.

## *Pre-Behavioral Studies Showing Orthogonality*

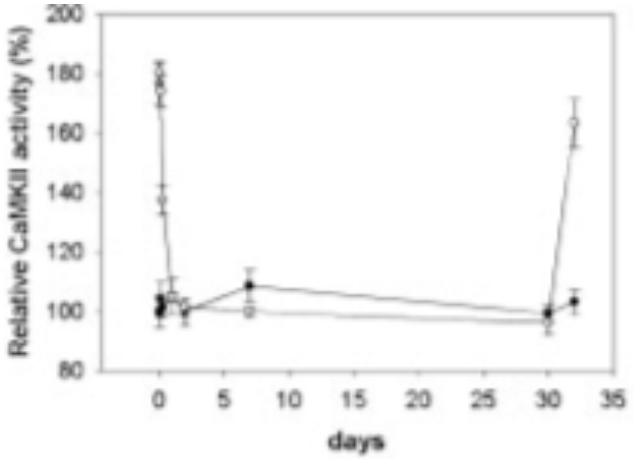
- Mutant kinase localized to specific regions under study




- Orthogonal inhibition




- Overexpressed kinase activity can be inhibited and reversible

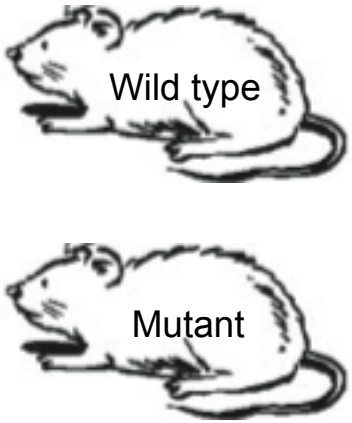



*in vivo*

Shokat, K. M.; Tsien, J. Z.; *et al. Proc. Natl. Acad. Sci. USA* **2003**, *100*, 4287.

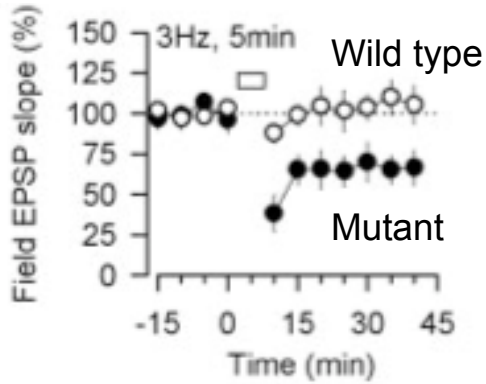
## *Pre-Behavioral Studies Showing Electrophisiological Significance*

- Significant long term depression and bidirectional shifting for kinase mutant mice was encouraging

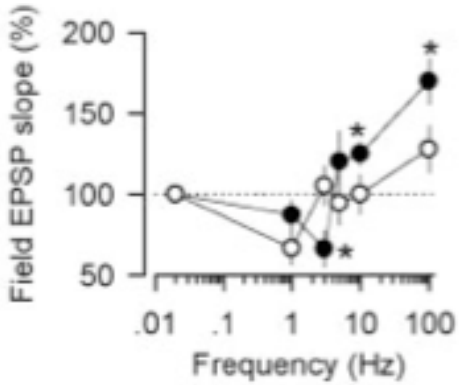



Sacrifice

synaptic electrophisiology of hippocampal slices

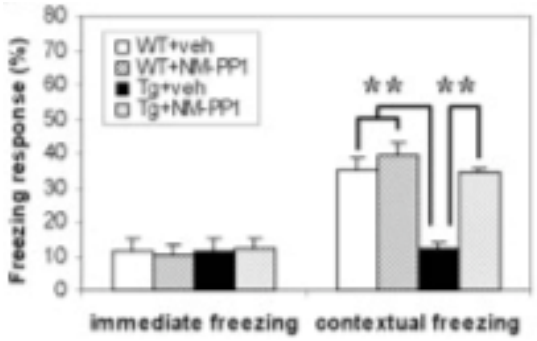



Long Term Depression




Bidirectional Plasticity Response Curve

- NM-PP1 (**1**) inhibitor rescues fear-based freezing response




*in vivo*

Shokat, K. M.; Tsien, J. Z.; *et al. Proc. Natl. Acad. Sci. USA* **2003**, *100*, 4287.

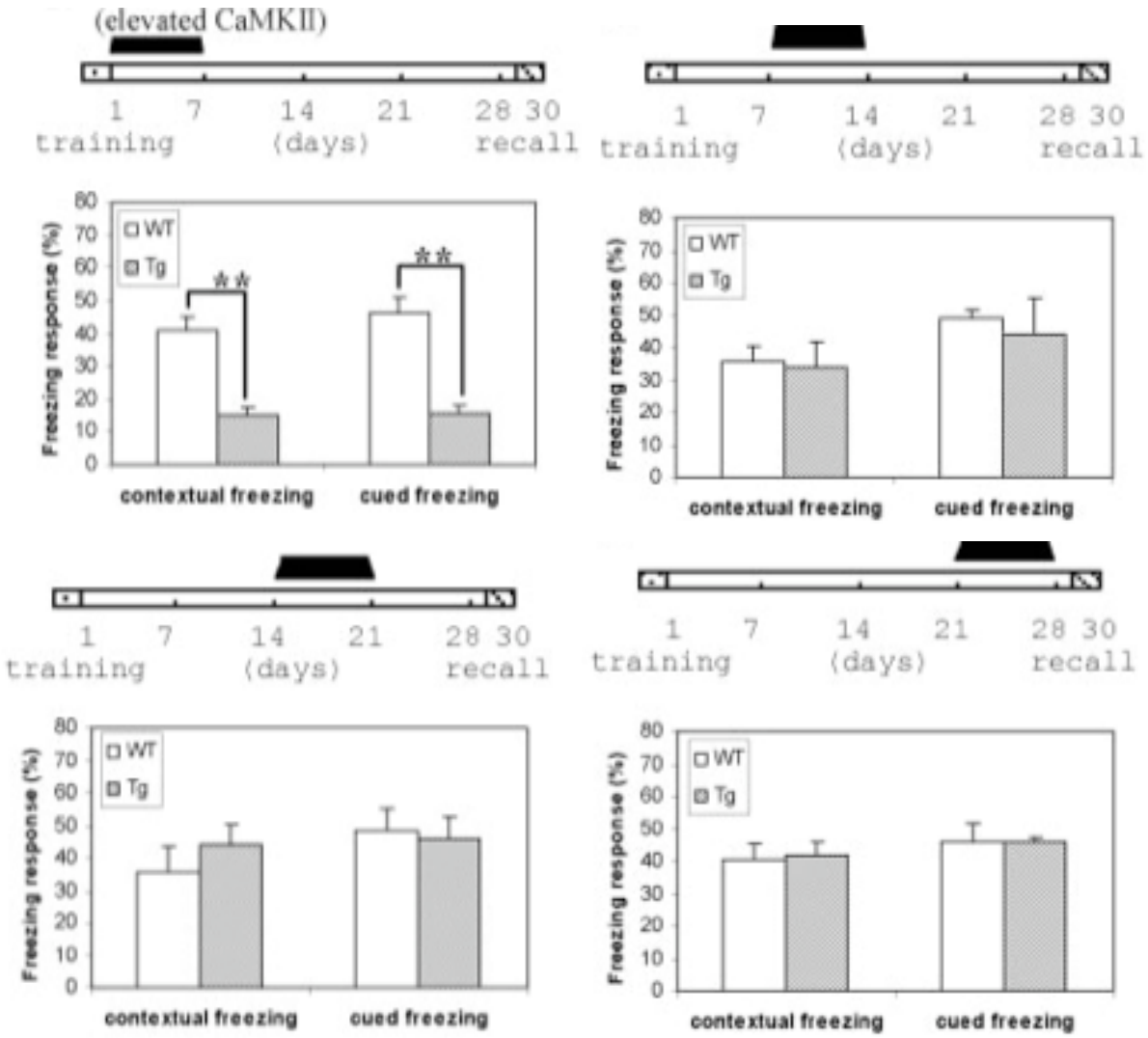
## *Behavioral Studies: Temporal Response Crucial for Research and Discovery*

- Fear-based learning for this mechanism is limited to the first week of post-trauma

CaMKII involved in learning by Calcium channel activity

Each of 4 mice allowed elevated CaMKII levels in a different week (rest supressed by NM-PP1 inhibitor

Because elevated CaMKII levels only dampen fear response in first week, contenxtual and cued response limited to that week




*in vivo*

Shokat, K. M.; Tsien, J. Z.; *et al. Proc. Natl. Acad. Sci. USA* **2003**, *100*, 4287.

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Shokat, K. M. *et. al. Nature* **2000**, *407*, 395.

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Kwak, E. L.; *et al. Proc. Natl. Acad. Sci. USA* **2005**, *102*, 7665.
Patil, C., Walter, P. *Curr. Opin. Cell Biol.* **2001**, *13*, 349;
Papa, F. R., Zhang, C., Shokat, K. M.; Walter, P. *Science* **2003**, *302*, 1533.
Crackower, M. A. *et al. Cell* **2002**, *110*, 737; Patrucco, E. *et al. Cell* **2004**, *118*, 375.
Bi, L.; *et al. Mamm. Genome* **2002**, *13*, 169.

PP1 inhibitors

Knight, Z. A.; Shokat, K. M. *Cell* **2007**, *123*, 425.

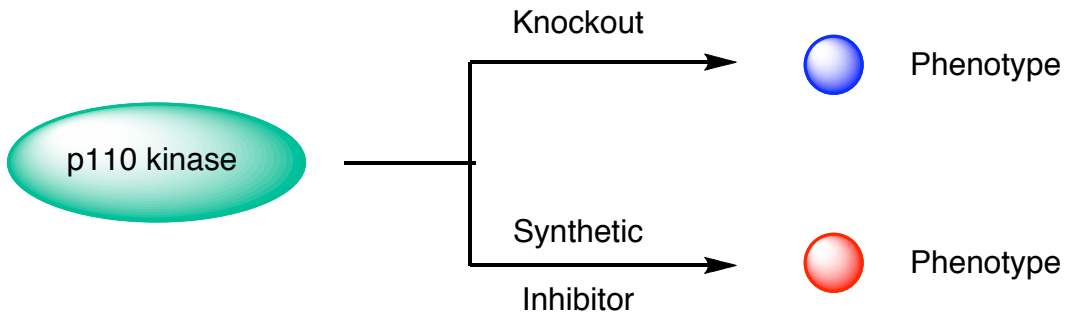
Shokat, K, M,; *et al. Proc. Natl. Acad. Sci. USA* **1997**, *94*, 3565.
Shah, K.; Shokat, K. M. *Chem. Biol.* **2002**, *9*, 35.
Shokat, K, M,; *et al. Proc. Natl. Acad. Sci. USA* **2008**, *105*, 1442.
Maly, D. J.; Allen, J. A.; Shokat, K. M. *J. Am. Chem. Soc.* **2004**, *126*, 9160.

A*TP analogs

Shokat, K. M.; Tsien, J. Z.; *et al. Proc. Natl. Acad. Sci. USA* **2003**, *100*, 4287.

## *Kinase Inhibition that Leaves Protein Function Intact*

- Small molecule inhibitors and knockouts of the same protein produce different phenotypes




Crackower, M. A. *et al. Cell* **2002**, *110*, 737; Patrucco, E. *et al. Cell* **2004**, *118*, 375.

## *Kinase Inhibition that Leaves Protein Function Intact*

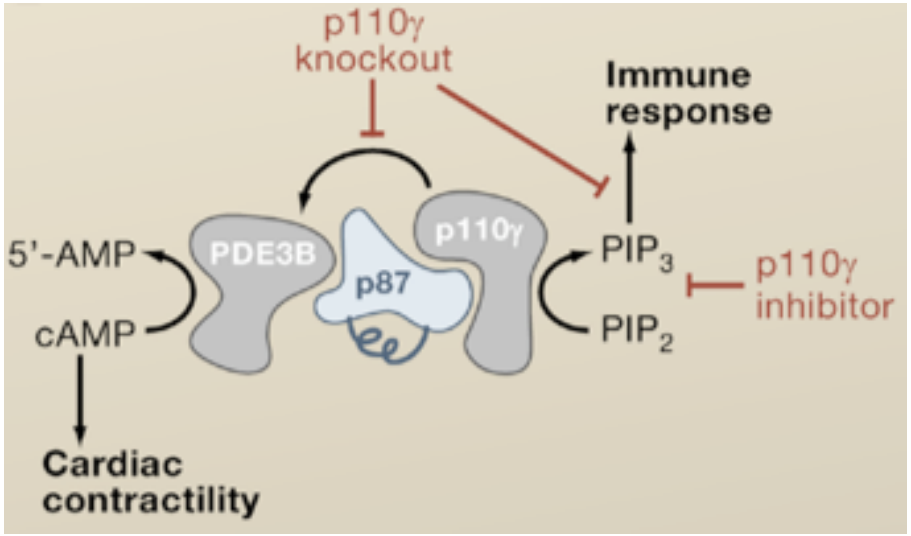
- Small molecule inhibitors and knockouts of the same protein produce different phenotypes
- p110γ mediates leukocyte reaction to the inflammatory response; mice lacking it have more dampened immune response, leading to the search for anti-inflammatory drugs

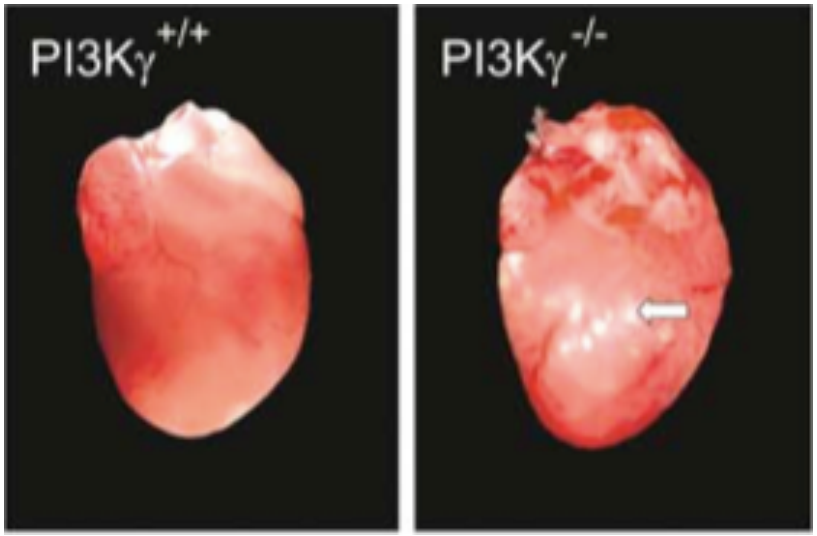

P110γ $^{-/-}$ (knockout) Reduced immune response

Crackower, M. A. *et al. Cell* **2002**, *110*, 737; Patrucco, E. *et al. Cell* **2004**, *118*, 375.

## *Kinase Inhibition that Leaves Protein Function Intact*

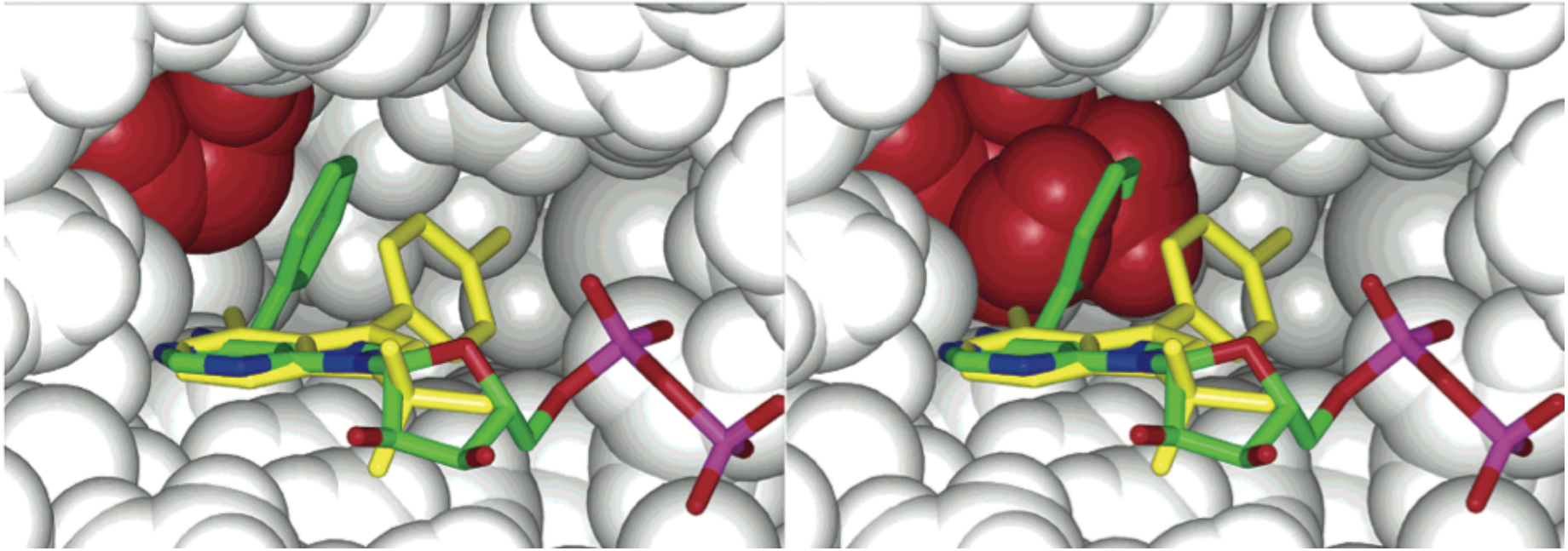
- Small molecule inhibitors and knockouts of the same protein produce different phenotypes
- p110γ mediates leukocyte reaction to the inflammatory response; mice lacking it have more dampened immune response, leading to the search for anti-inflammatory drugs
- While the p110γ knockout mice had elevated myocardial damage from aortic constriction, the mice with overexpressed kinase-dead (mutant) p110γ protects them from this damage
- Why does p110γ deletion produce a different phenotype from kinase-dead overexpression?
- Knockin mice with kinase-dead levels at wild-type concentration retain immune deficit but with normal heart tissue --> cardiac effect not due to loss of kinase activity
- p110g allosterically binds to an enzyme that catalyzes cAMP destruction ([cAMP] proportional to pathological cardiac response); knockouts prevent this, inhibitors miss the point



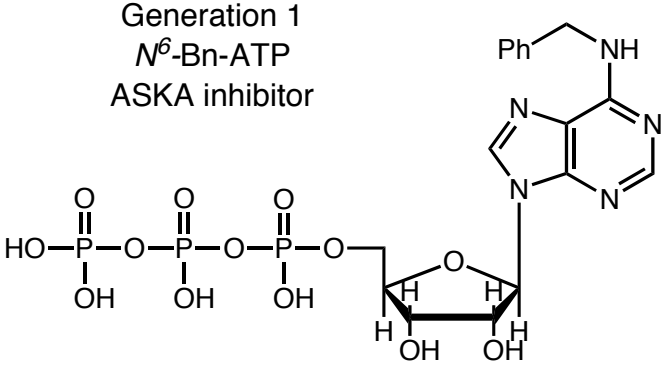

Crackower, M. A. *et al. Cell* **2002**, *110*, 737; Patrucco, E. *et al. Cell* **2004**, *118*, 375.

# *Structural Analysis Leads to a Rationally Designed ASKA Inhibitor*



- Shokat noticed naturally occuring hydrophobic pocket to be exploited, requiring a new inhibitor
- New inhibitor desing challenging: "requirements for substrate recognition/transition state stabilization different versus inhibitor binding at the same active site."

Generation 1
$N^6$-Bn-ATP
ASKA inhibitor



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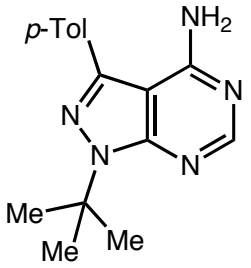
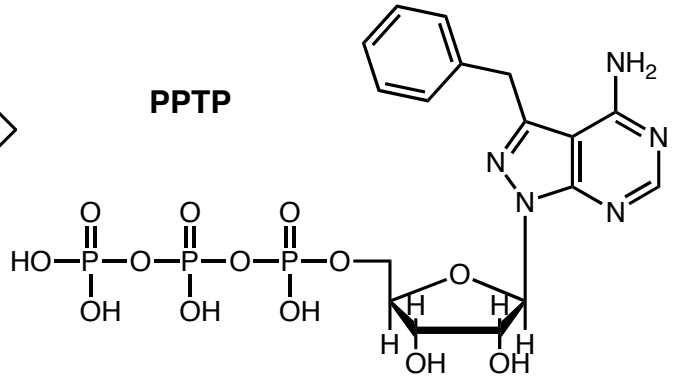


**PP1**: Known
kinase inhibitor



**PPTP**



Kraybill, B. C.; Ilkin, L. L.; Blethrow, J. D.; Morgan, D. O.; Shokat, K. M. *J. Am. Chem. Soc.* **2002**, *124*, 12118.

# *An Apex of Traditional Pharmacology*

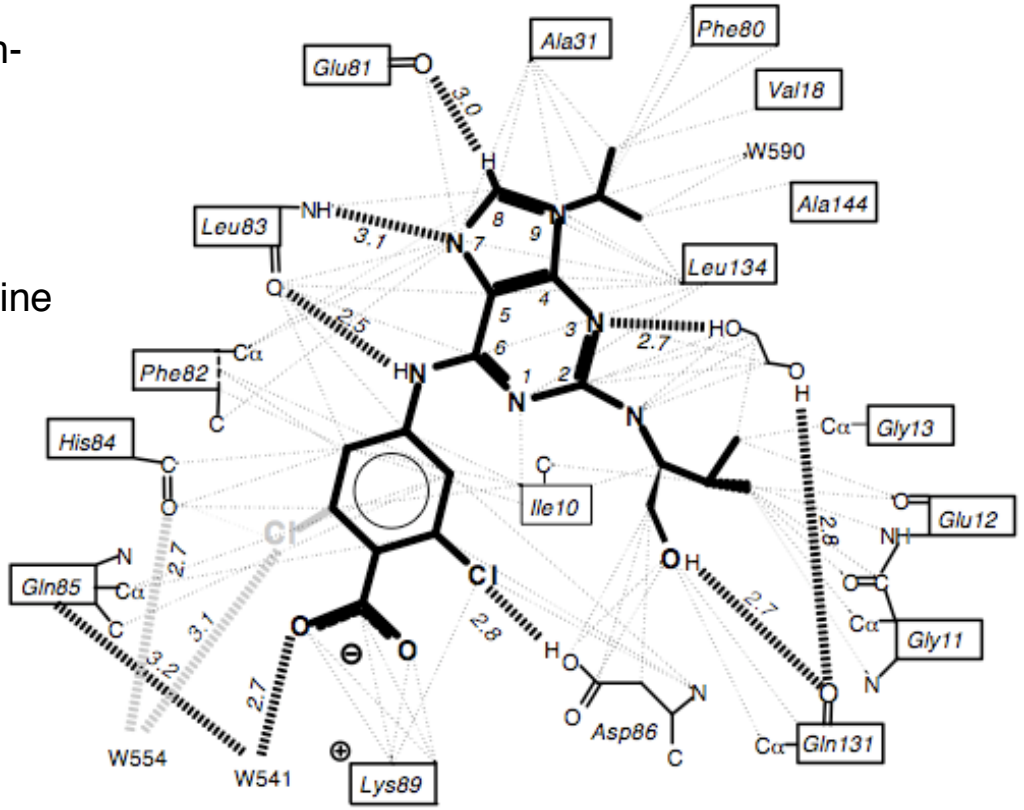
- Schultz and coworkers discover Purvalanol B, a potent and moderately selective inhibitor of cyclin dependent kinase (CDK2 in humans cdc28 in yiest)

- They use a large cellular based purine high-throughput 96-well plate screening technique

- Diversity of 2, 6 and 9 positions based on knowledge of co-crystal structure for olomoucine

Thin dashed lines:
van der Waals
Interactions

Thick dashed lines:
hydrogen bonds

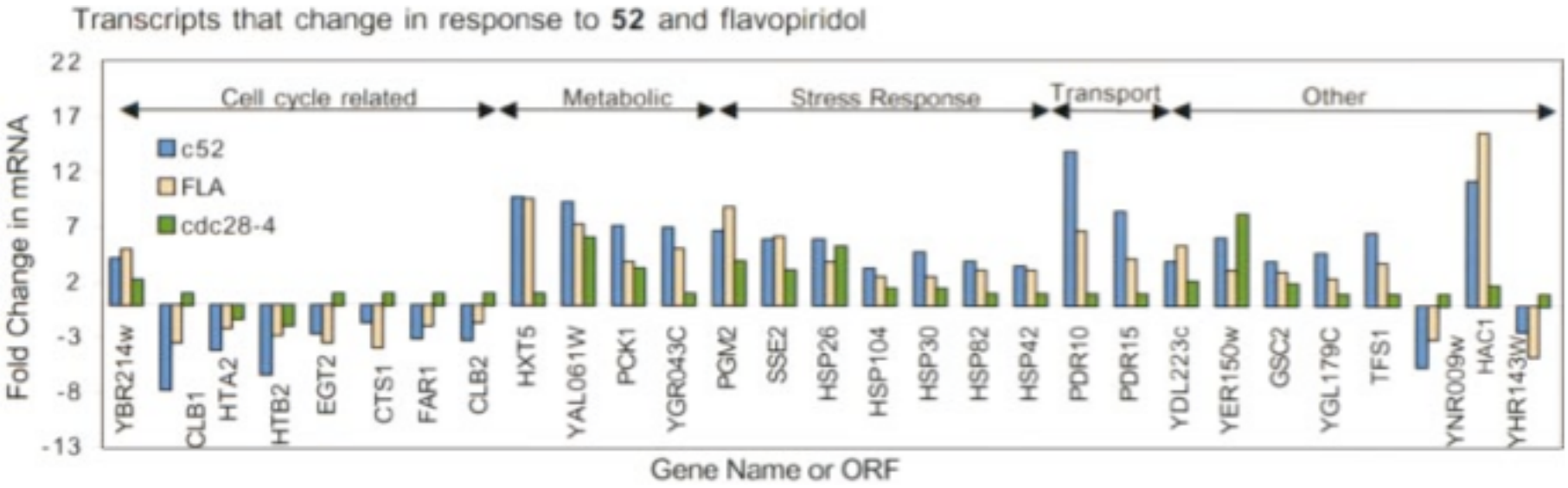



Schultz, P. G.; *et al. Science* **1998**, *281*, 533.

# *Traditional Pharmacology Has Limits*

- For the yeast case (cdc28) Schultz could measure mRNA levels of nearly all yeast genes as determined by high-density oligonucleotide expression arrays

- mRNA level changes are representative of the degree of up or down-regulation of that gene




- While discovering a potent and moderately selective new inhibitor of CDK2, and a wealth of information regarding yeast genetic up/down regulation, there was one drawback:

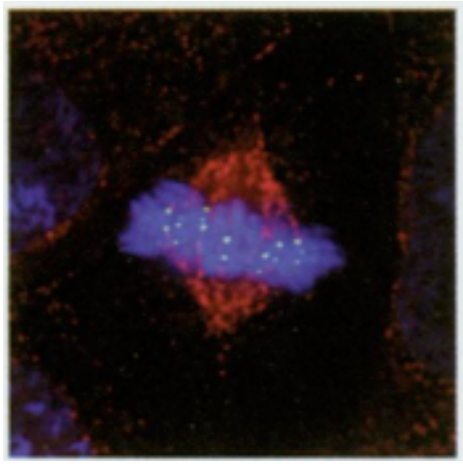
> "Our current experimental design does not allow us to definitively Identify the primary target or targets of inhibition by [purvalanol]."

Schultz, P. G.; *et al. Science* **1998**, *281*, 533.

## *The Scaffolding Function of Aurora B Kinase*

- Aurora kinases regulate spindle assembly and chromosomal alignment during mitosis
- Many tumors overexpress Aurora kinases which lead to interest in inhibitor development
- RNAi of Aurora B leads to major chromosomal alignment problems due to disruption of Aurora B interacting with Survivin at the centromere
- When RNAi treated Aurora B is then inhibited with ZM447439, Survivin is then localized correctly
- Therefore, the kinase is involved in a "scaffolding" effect not directly tied to tyrosine phosphorylation

Aurora B kinase —mitosis→



Ditchfield, C.; *et al. Cell Biol.* **2003**, *161*, 267.

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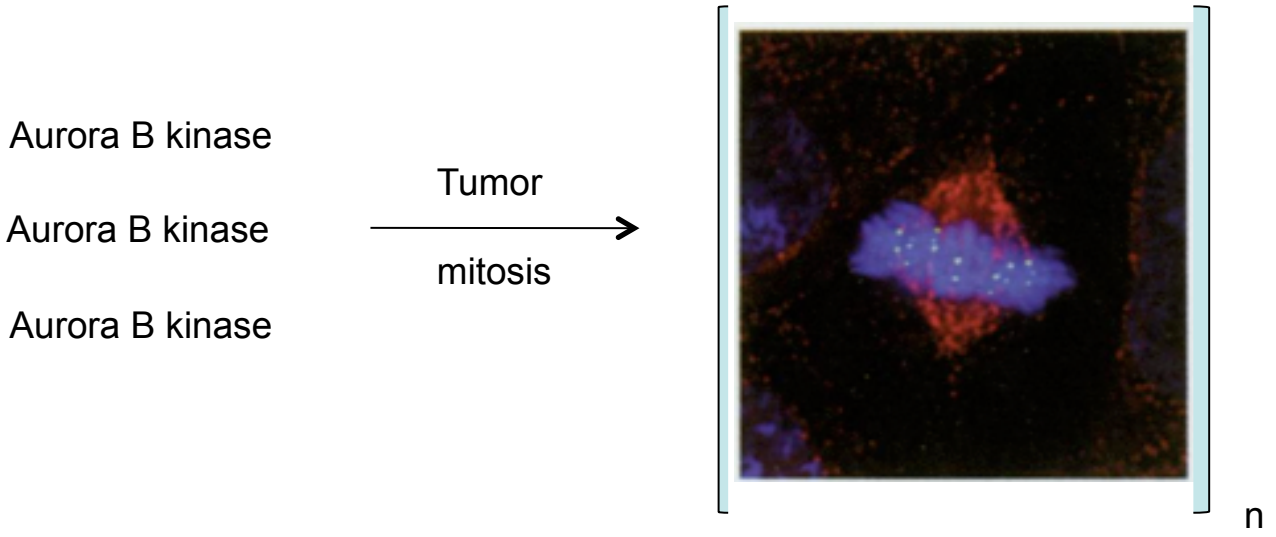


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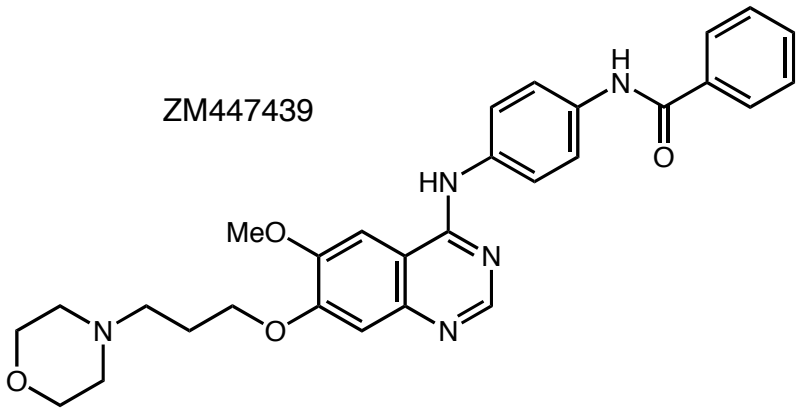
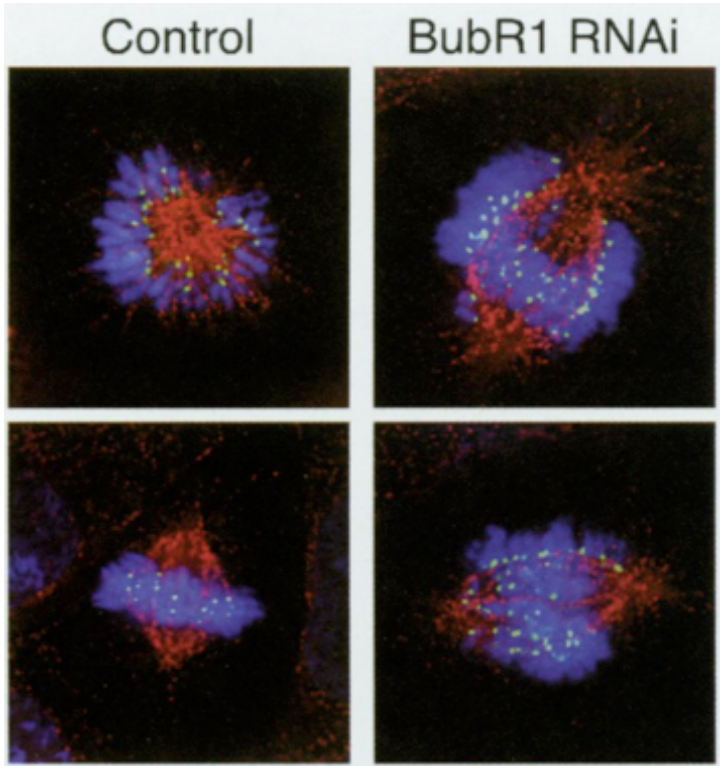
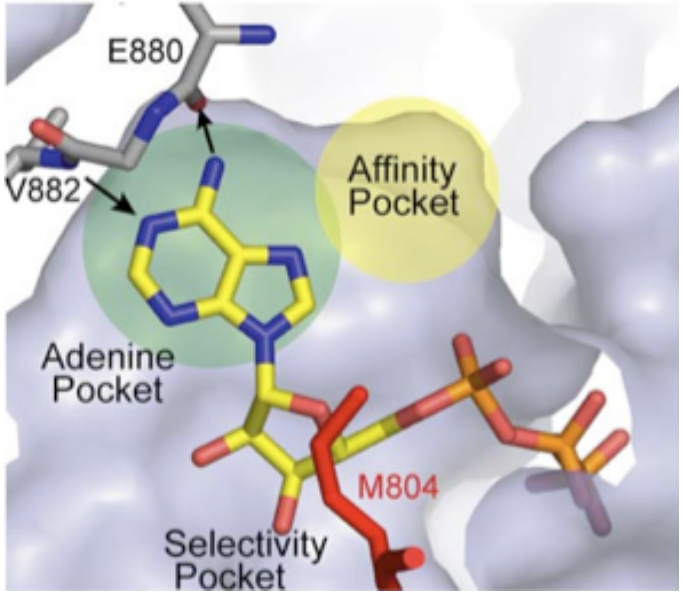


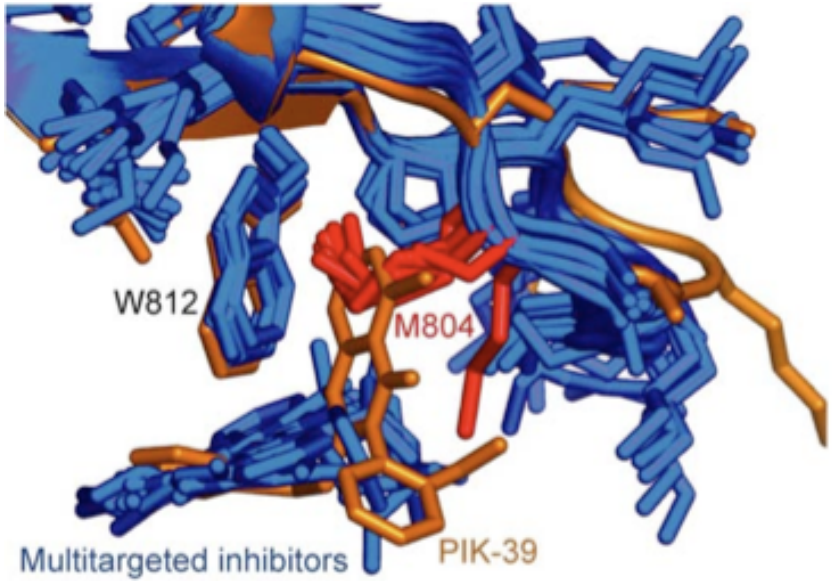




Ditchfield, C.; *et al. Cell Biol.* **2003**, *161*, 267.

## *Importance of Relative Enzyme Stoichiometry Masked by Knockout*

- Both PI3 kinases, p110α and p110β, carry signals from the growth factor, insulin
- Because knockout of either kinase isoform kills mice early in development we know that they cannot compensate for each other but don't know their roles
- Heterozygous deletion of either produces no phenotype; deletion of p85, the p110 binding partner, paradoxically increases insulin signaling
- Both knockin kinase-dead mice as well as mice treated with a p110α–selective inhibitor show reduced insulin signaling
- Paradox: why do mice lacking p110 or p85 show normal or increased insulin signaling while kinase-dead mutants (or their equivalent p110α-inhibited mice) show a reduction?
- Resulting Model: since p85 can function as a negative regulator of p110, insulin transduction controlled by relative stoichiometry of p110 to p85 rather than absolute amounts.

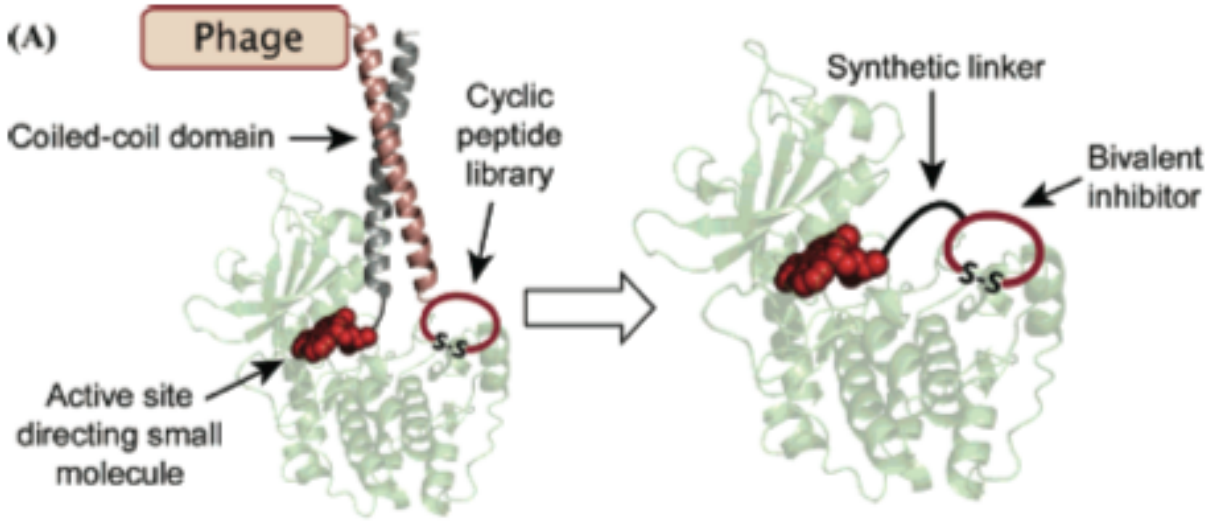



Shokat, K. M.; *et al. Cell* **2006**, *125*, 733.

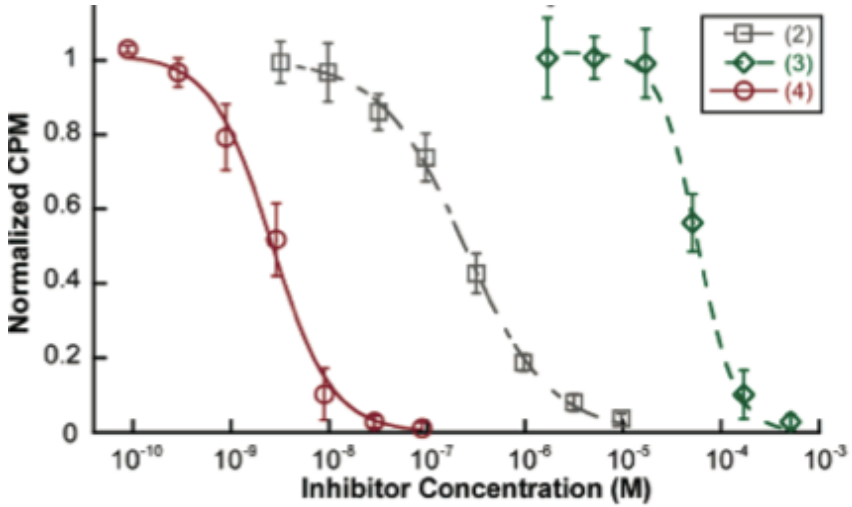
## *Tethering Small Molecules to a Phage-Display Library*

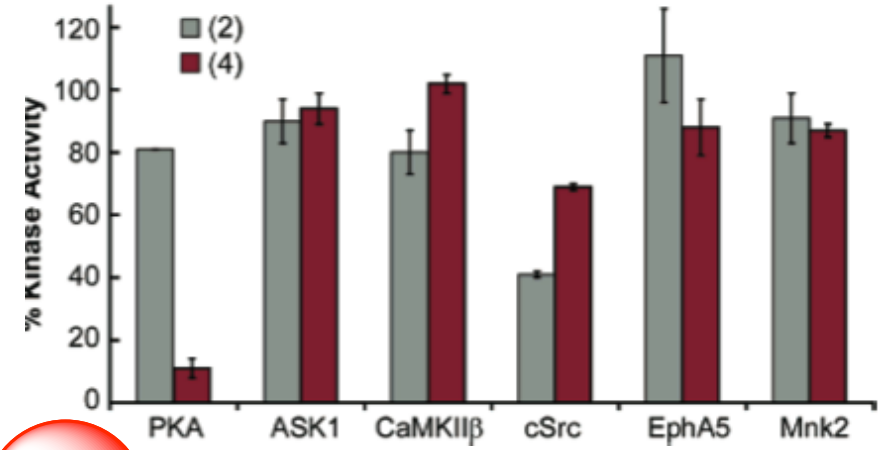
- If the phosphorylated protein target of a specific kinase is known, an abbreviated peptide analog of that protein can be synthetically appended to an ATP-competitive inhibitor.
- Ghosh used a phage display library approach to discover the right match.
- Phage display is a method for the study of protein-protein, protein peptide and DNA interactions that uses bacteriophages to connect proteins with the genetic information that encodes them



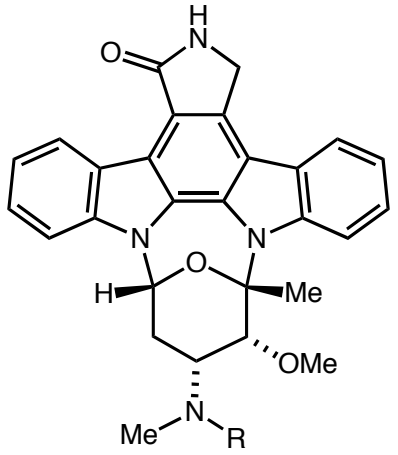
# *Bivalency and Synergystic Affinity and Selectivity*

■ By seeking a bivalent inhibitor, a synergistic binding with warhead and cyclic peptide was employed to acheive selective inhibition of cAMP-dependent protein Kinase A (PKA).

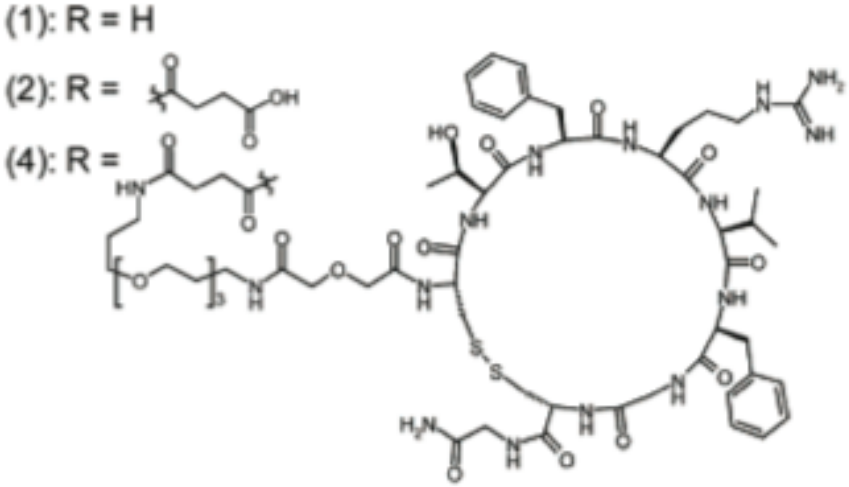



*in vitro*




staurosporine (R = H)




Meyer, S. C.; Shomin, C. D.; Gaj, T.; Ghosh, I. *J. Am. Chem. Soc.* **2007**, *129*, 13812.