Target validation

A chemical switch for inhibitor-sensitive alleles of any protein kinase

Anthony C. Bishop*, Jeffrey A. Ubersax†, Dejah T. Petsch‡, Dina P. Matheos§, Nathanael S. Gray‖, Justin Blethrow†, Eiji Shimizu§, Joe Z. Tsien§, Peter G. Schultz‖, Mark D. Rose§, John L. Wood‡, David O. Morgan† & Kevan M. Shokat*¶

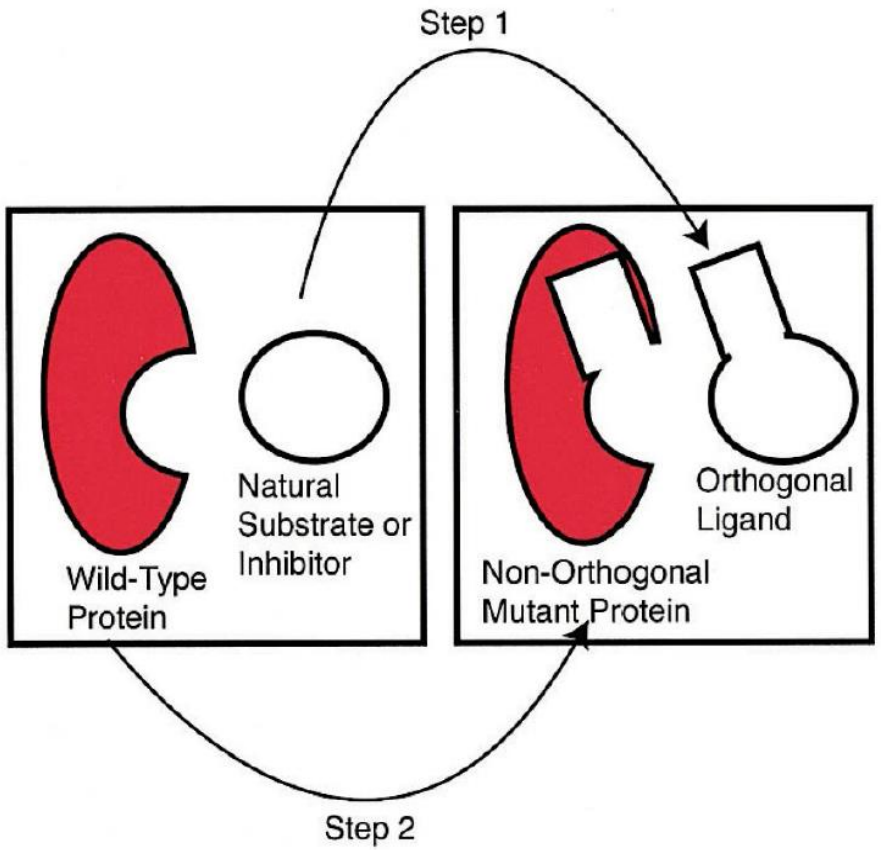
* *Department of Chemistry, Princeton University, Princeton, New Jersey 08544, USA*
† *Departments of Physiology and Biochemistry & Biophysics, University of California San Francisco, San Francisco, California 94143-0444, USA*
‡ *Department of Chemistry, Yale University, New Haven, Connecticut 06520-8107, USA*
§ *Department of Molecular Biology, Princeton University, Princeton, New Jersey 08544, USA*
‖ *Genomics Institute of the Novartis Research Foundation, San Diego, California 92121, USA*

Protein kinases have proved to be largely resistant to the design of highly specific inhibitors, even with the aid of combinatorial chemistry[1]. The lack of these reagents has complicated efforts to assign specific signalling roles to individual kinases. Here we describe a chemical genetic strategy for sensitizing protein

Relevant for exam: Figures 1, 2 and SI Figure showing staurosporine structure (a) and K252a data (b)

«Bump and hole» strategy

Generating “orthogonal” ligand-protein pairs/ allele-specific inhibitors



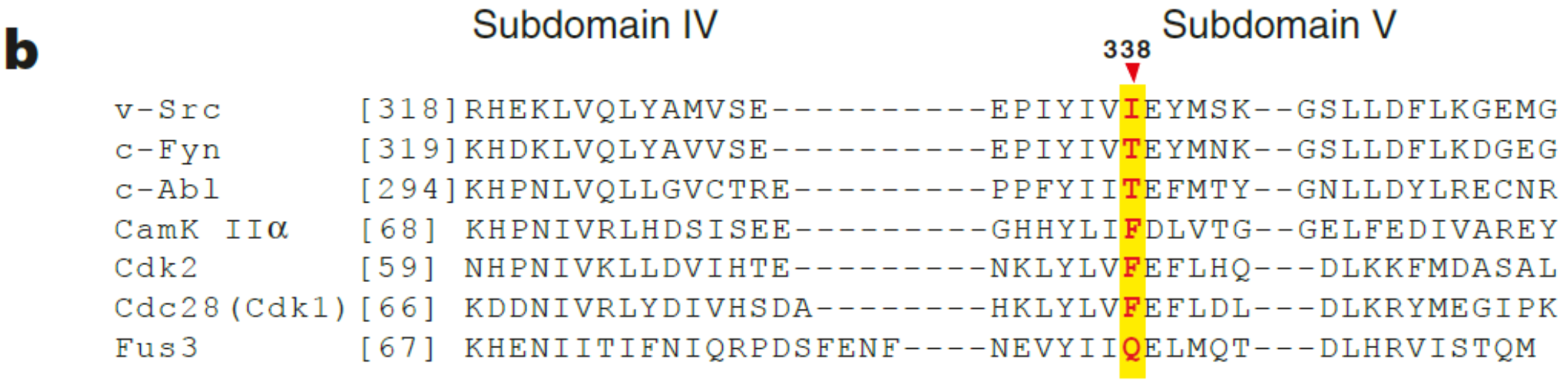
Annu. Rev. Biophys. Biomol. Struct. 2000. 29:577–606

Figure 1a

a

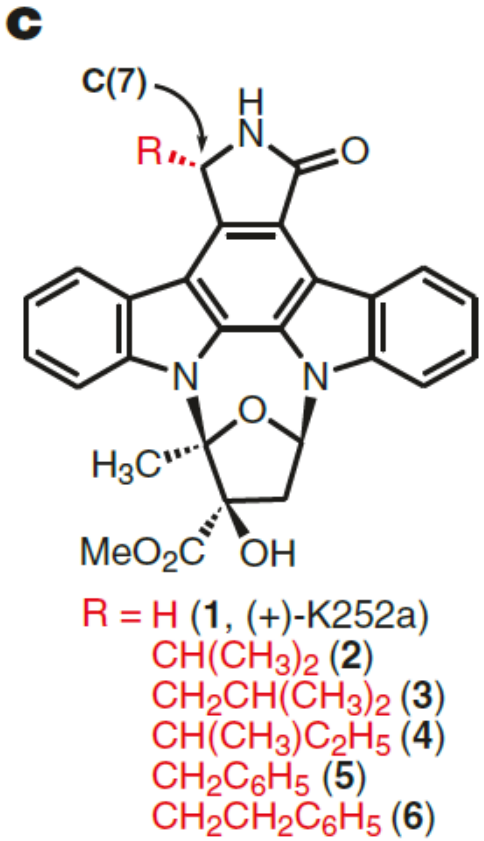
Protein kinase	Kinase family	Specificity	Cellular function	% identity to v-Src
v-Src	Src	Tyr	oncogenic transformation	100
c-Fyn	Src	Tyr	lymphocyte activation	84
c-Abl	Abl	Tyr	F-actin binding, transcription	51
CAMK IIα	calcium/calmodulin dependent	Ser/Thr	long-term potentiation, memory	26
CDK2	cyclin dependent	Ser/Thr	mammalian cell-cycle progression	27
Cdc28	cyclin dependent	Ser/Thr	*S. cerevisiae* cell-cycle progression	27
Fus3	mitogen-activated	Ser/Thr	*S. cerevisiae* mating	28

Figure 1b



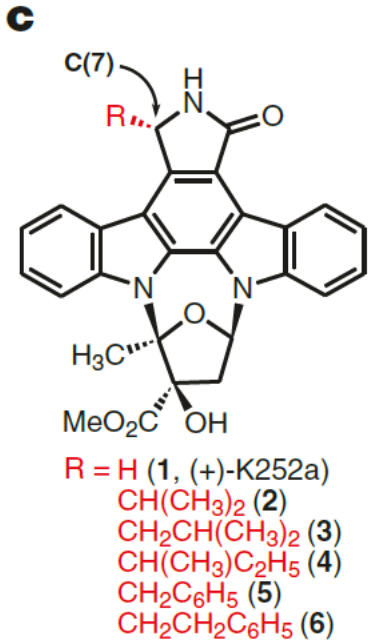
- Why mutation of position 338?
- Mutation to which amino acid?
- Was this strategy applied successfully before? To which enzyme?

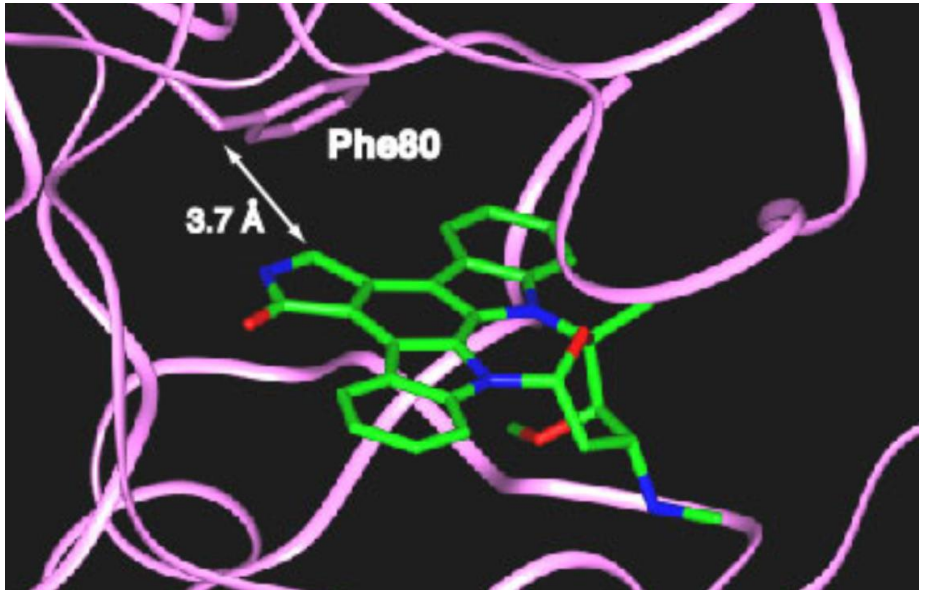
Figure 1c



- Why was the molecule K252a used?
- Why modification at position C(7)?
- How did they know the binding orientation of K252a?
- Why trying five different modifications?

Supplementary figure (CDK2/staurosporine)



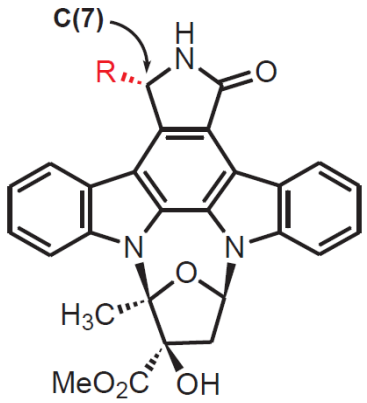


CDK2

- Why mutation of position 80 and not position 338?

Supplementary figure (K252a activity)

c



R = H (**1**, (+)-K252a)
$CH(CH_3)_2$ (**2**)
$CH_2CH(CH_3)_2$ (**3**)
$CH(CH_3)C_2H_5$ (**4**)
$CH_2C_6H_5$ (**5**)
$CH_2CH_2C_6H_5$ (**6**)

K252a

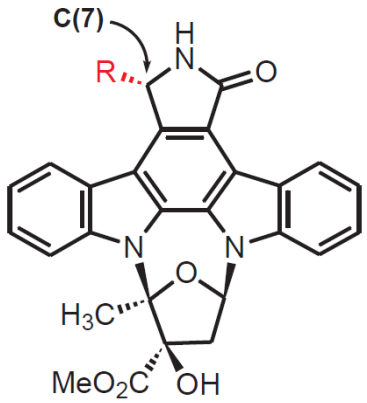
Compound / Kinase	1	3	6
v-Src	0.020	>33	12
c-Fyn	0.17	>33	>33
c-Abl	2.2	>33	>33
CDK2	0.020	22	3.7
CAMK II	0.0054	1.7	0.54

- Are the wild-type kinases inhibited by K252a variants 3 and 6?
- Are the kinase mutants inhibited?

Supplementary figure (K252a activity)

K252a

c



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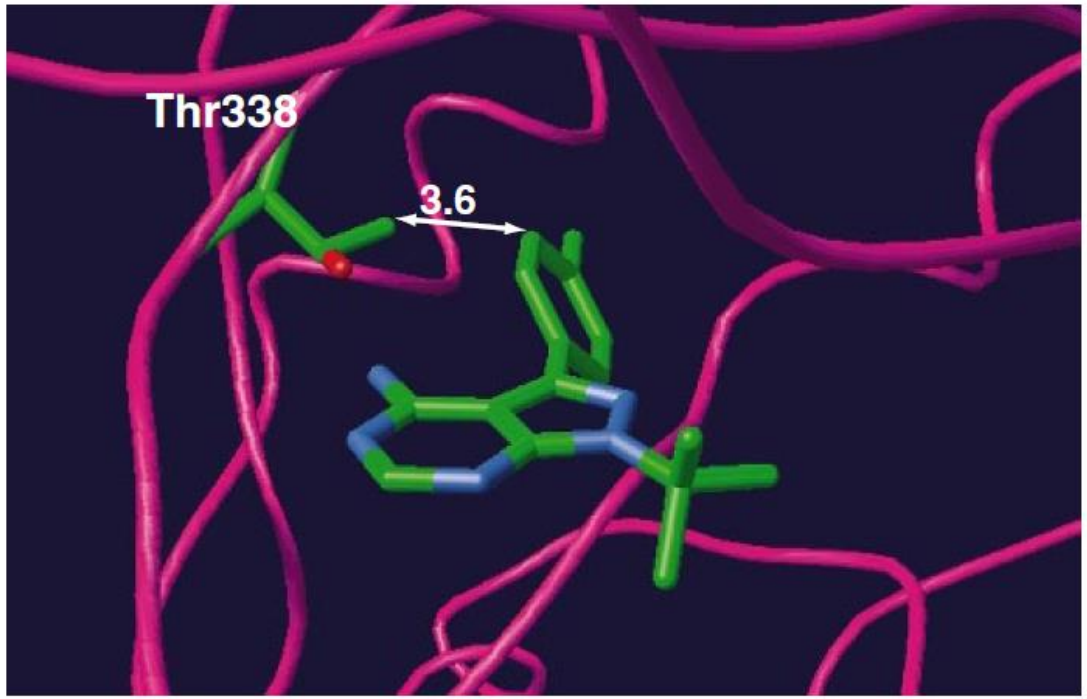
c-Abl	2.2	>33	>33
CDK2	0.020	22	3.7
CAMK II	0.0054	1.7	0.54
v-Src-as1		0.00023	0.0023
c-Fyn-as1		0.00055	0.0043
c-Abl-as2		3.1	9.6
CDK2-as1		0.070	0.037
CAMKII-as1		0.95	0.0040

- What does «as1» mean?
- Which selectivity was achieved for the inhibition of v-Src kinase?
- Which selectivity was achieved for the inhibition of CDK2 kinase?

Figure 1d

d

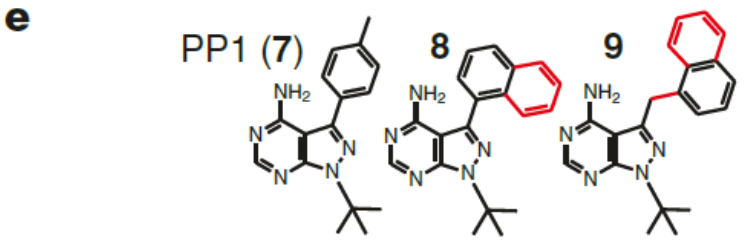


Hck

- What does this structure show?
- What does the «arrow» show?

Figure 1e

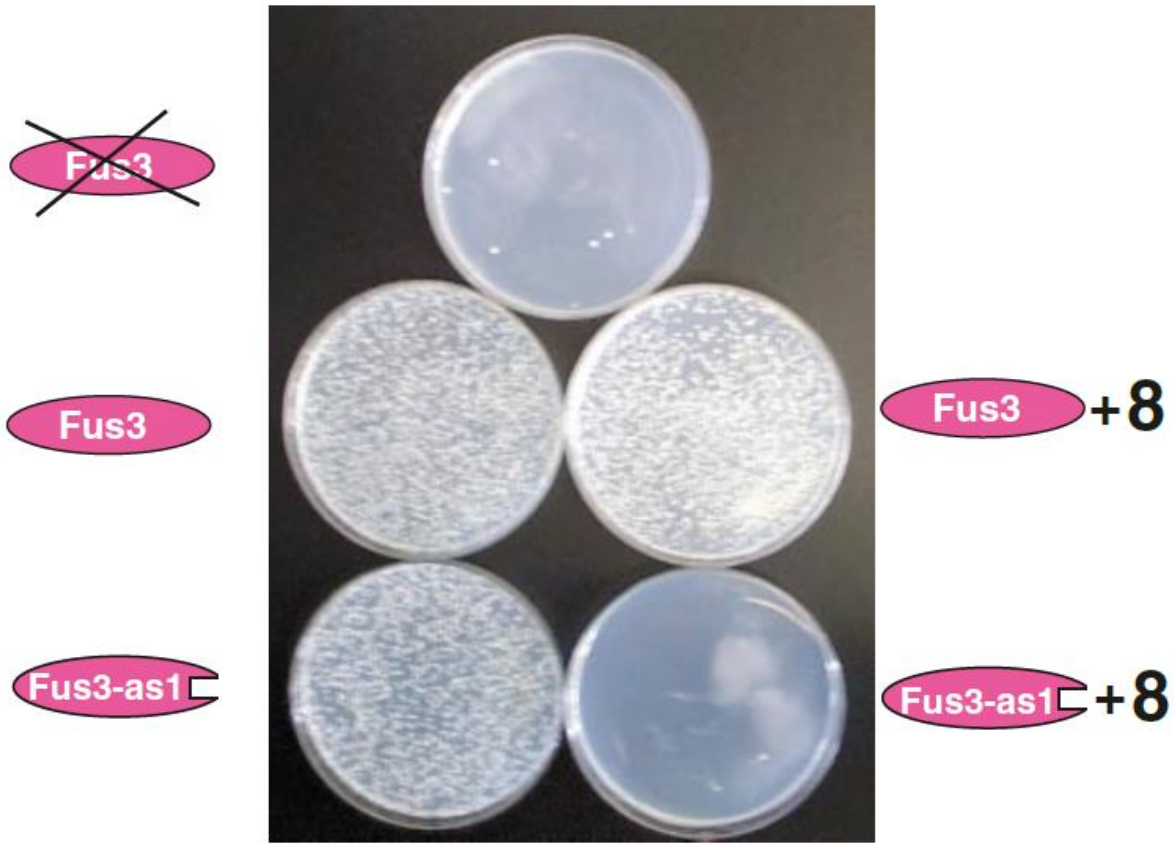
e



	PP1 (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

- Are the wild-type kinases inhibited by PP1 (8) and PP1 (9)?
- Are the mutants inhibited?
- Which selectivity was achieved for the inhibition of v-Src kinase?
- Which selectivity was achieved for the inhibition of CDK2 kinase?

Figure 2



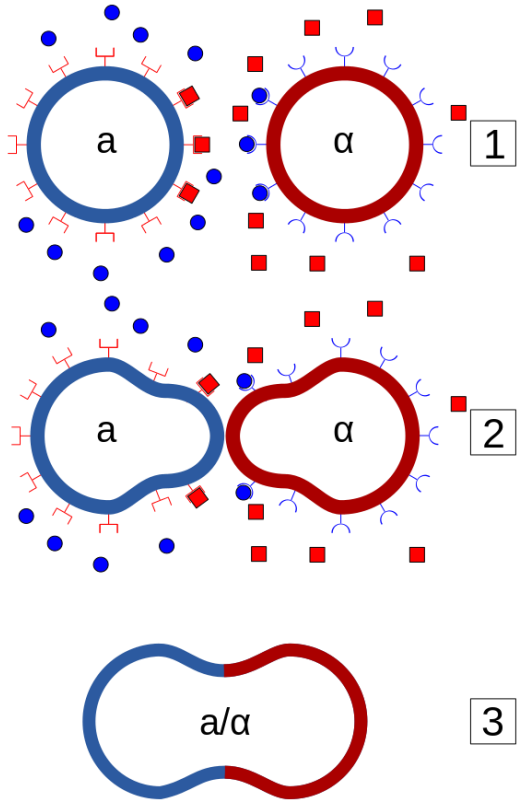
- What is Fus3?
- What is a «yeast mating» experiment
- How can «mated» yeast be identified experimentally? Why were ΔURA and Δ His strains used?
- Why are there almost no colonies on the plate «~~Fus3~~»
- Is the result for the lower four plates as expected?

Fus3

- Induction of pheromone-inducible genes
- Cell cycle arrest
- Cell fusion during yeast mating

No temperature sensitive mutant is available!

Yeast cell mating



Fus3 is required for cell fusion during yeast mating

Yeast cell mating

Mating haploid cells:

Wild-type or Fus mutant cells (URA3 *his3*) X Wild-type or Fus mutant cells (*fus1Δfus2Δura3Δ* HIS3)

Growth on media lacking uracil and His →
Selection for diploid cells

Figure 3a

a

Kinase	ATP kinetics			IC_{50} (μM 9)	
	K_M (μM)	k_{cat} (min^{-1})	k_{cat}/K_M ($\mu M^{-1}min^{-1}$)	@ 10 μM ATP	@ 1 mM ATP
Cdc28/Clb2	35	132	3.730	22	44
Cdc28-as1/Clb2	322	21.3	0.066	.0020	.0029

- What is Cdc28? Why did they choose to work with it?
- Is the kinase mutant «as1» as active as the wild-type?
- Which inhibitor was used? How selective is it?

Cdc28

Application to Cdc28

- Principle CDK in budding yeast
- Essential for progression through cell cycle
- Studied by termperature-sensitive mutants
- At 37 °C, mutants arrest in G1
- Is Cdc28 also involved in G2/M transition?

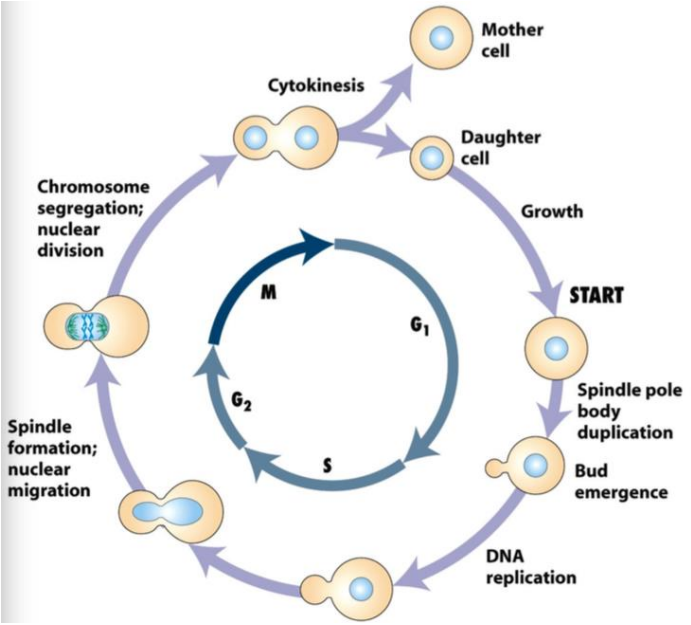
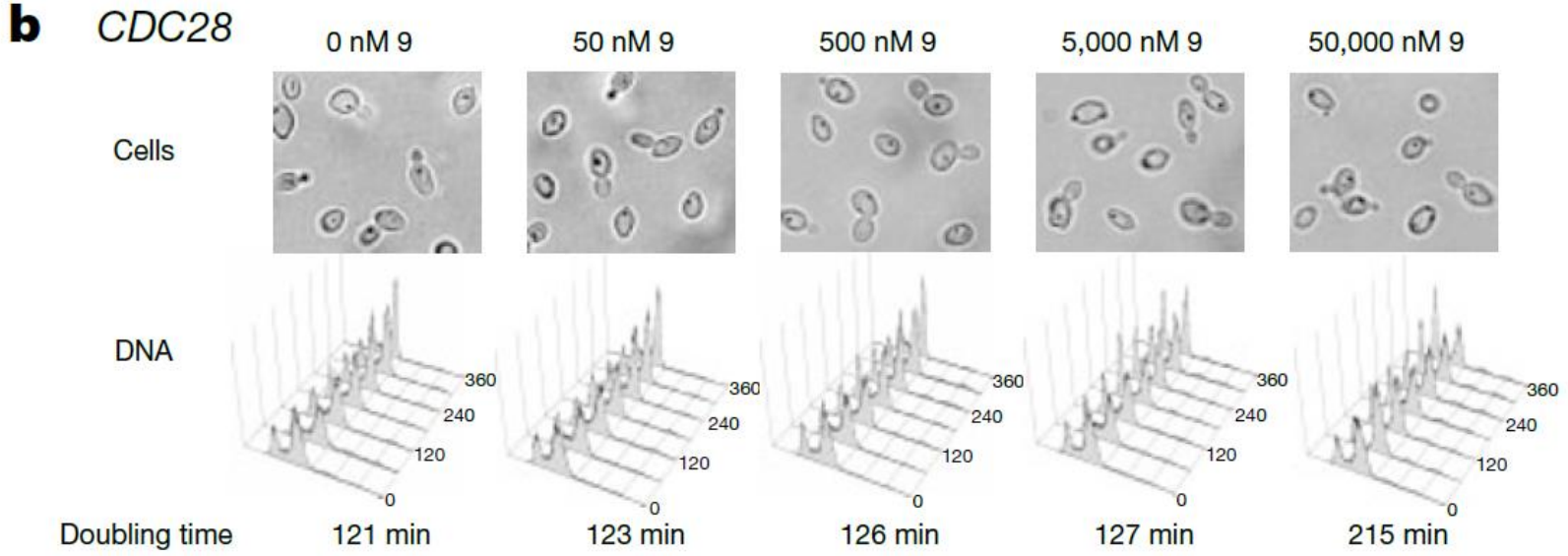
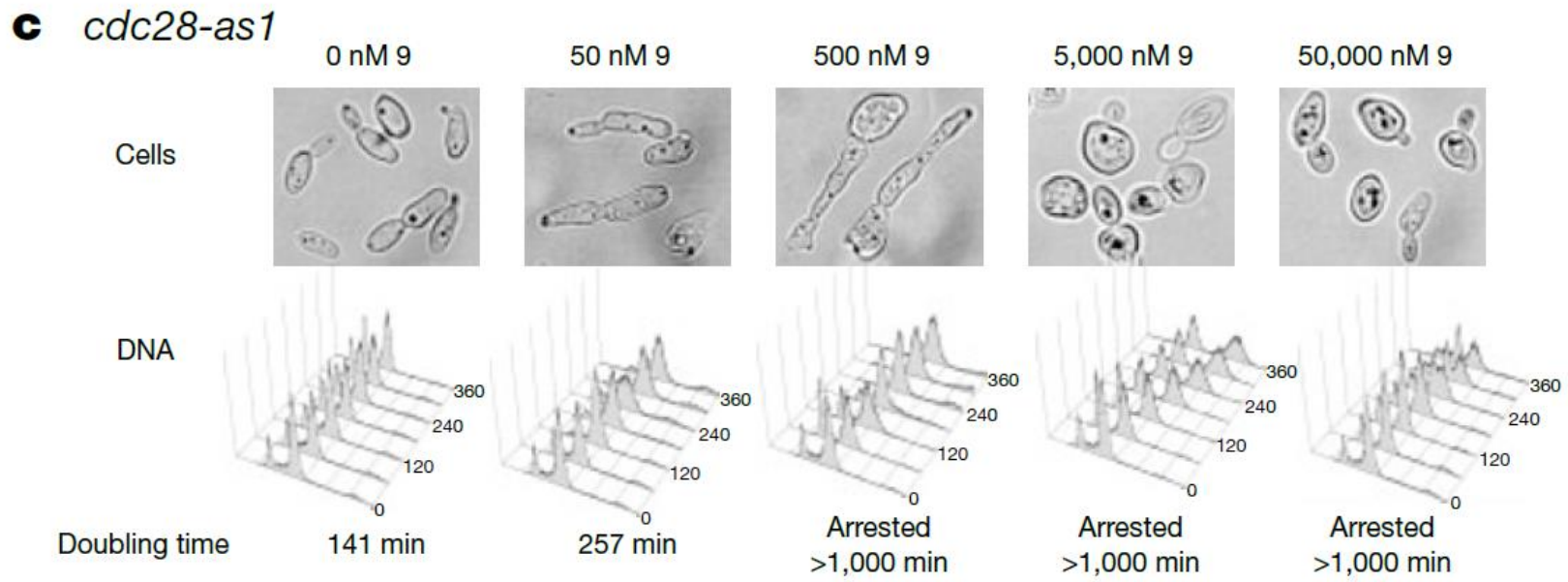


Figure 3b



- What do these experimental data show?

Figure 3c



- What do these experimental data show?
- Is the «knob and hole» strategy working here?

Figure 4a

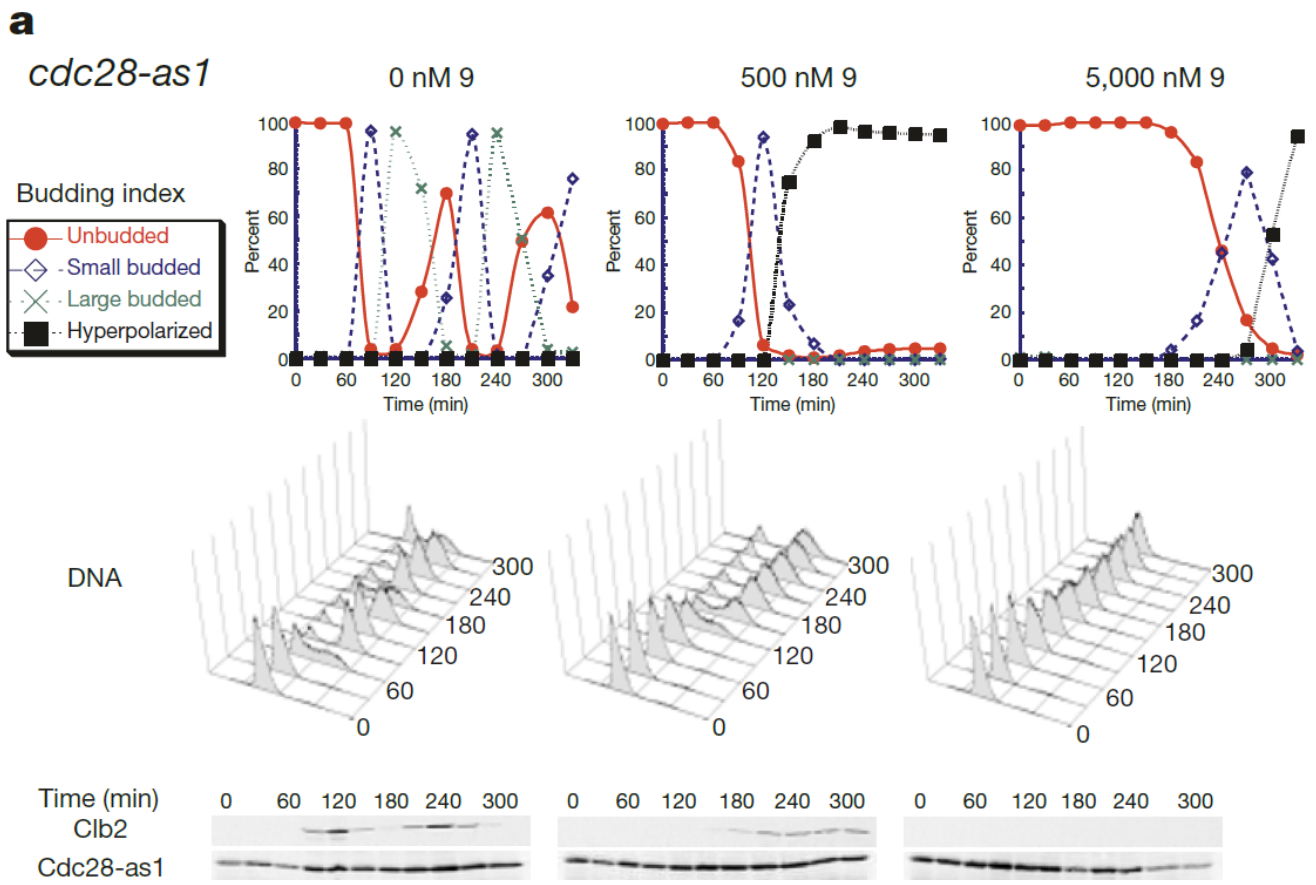

a
cdc28-as1
0 nM 9
500 nM 9
5,000 nM 9
Budding index
Unbudded
Small budded
Large budded
Hyperpolarized
Percent
Time (min)
DNA
Time (min)
Clb2
Cdc28-as1

Figure 4b

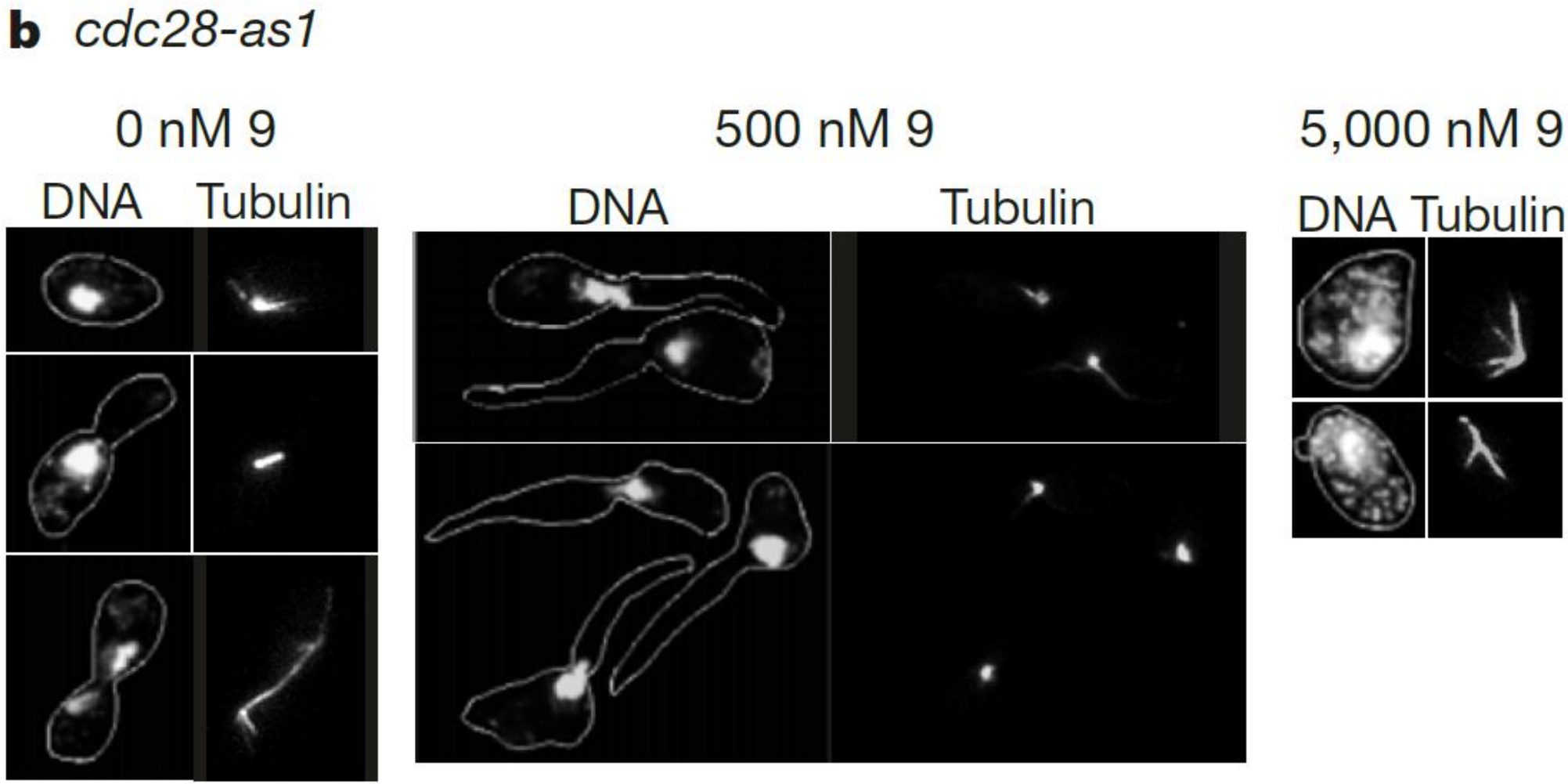
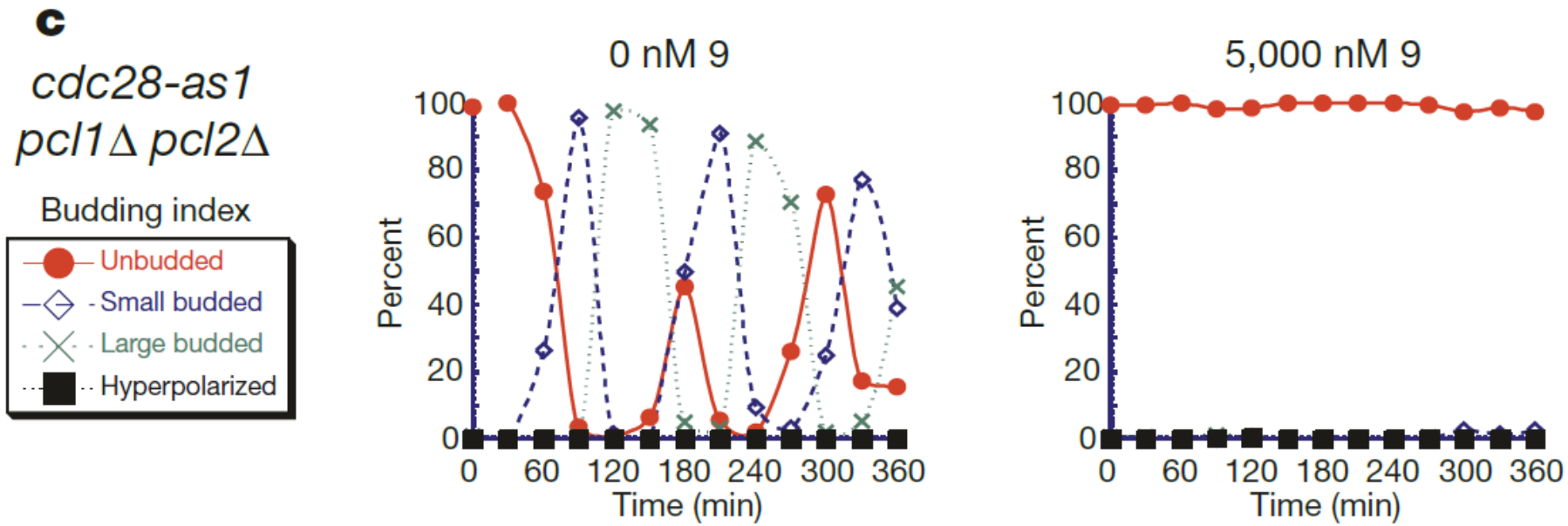

b cdc28-as1
0 nM 9
DNA
Tubulin
500 nM 9
DNA
Tubulin
5,000 nM 9
DNA Tubulin

Figure 4c


c
cdc28-as1
pcl1Δ pcl2Δ
Budding index
Unbudded
Small budded
Large budded
Hyperpolarized
0 nM 9
5,000 nM 9
Percent
Time (min)
0
20
40
60
80
100
0
60
120
180
240
300
360