Dynamic changes of Cdk1 signals during the budding yeast cell cycle

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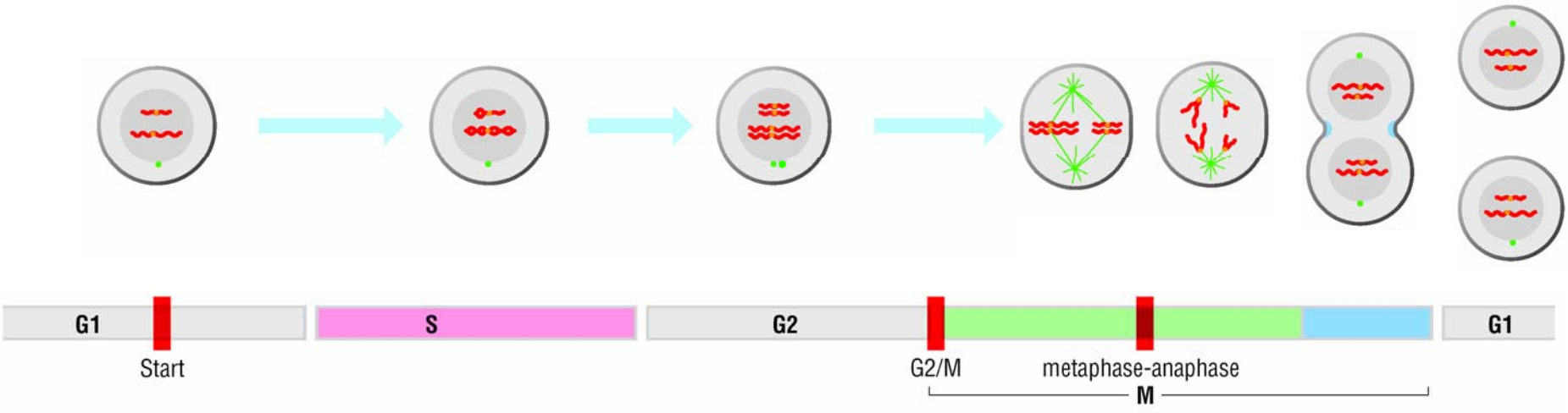
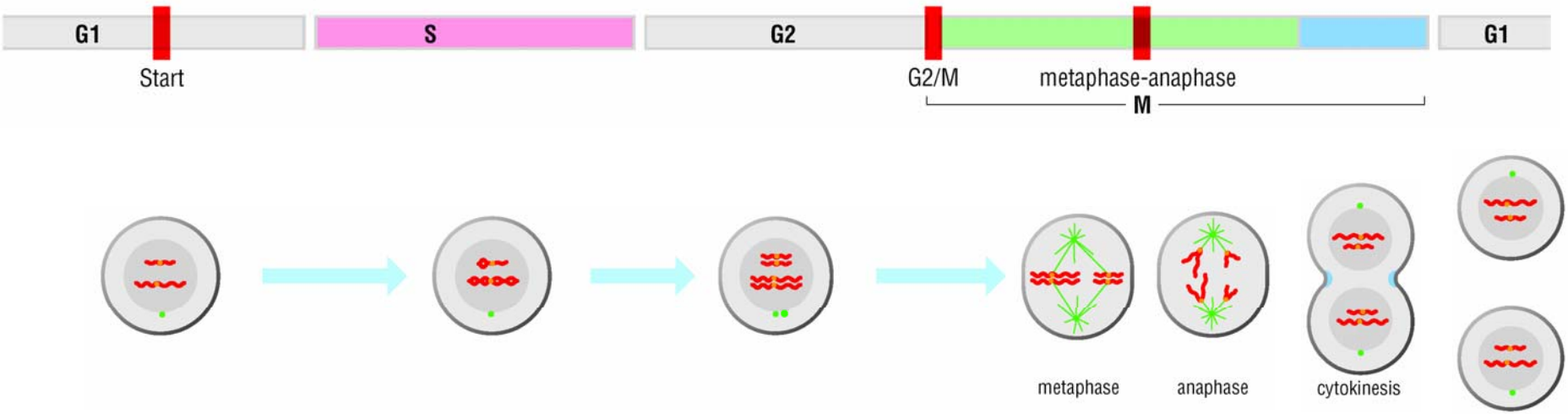
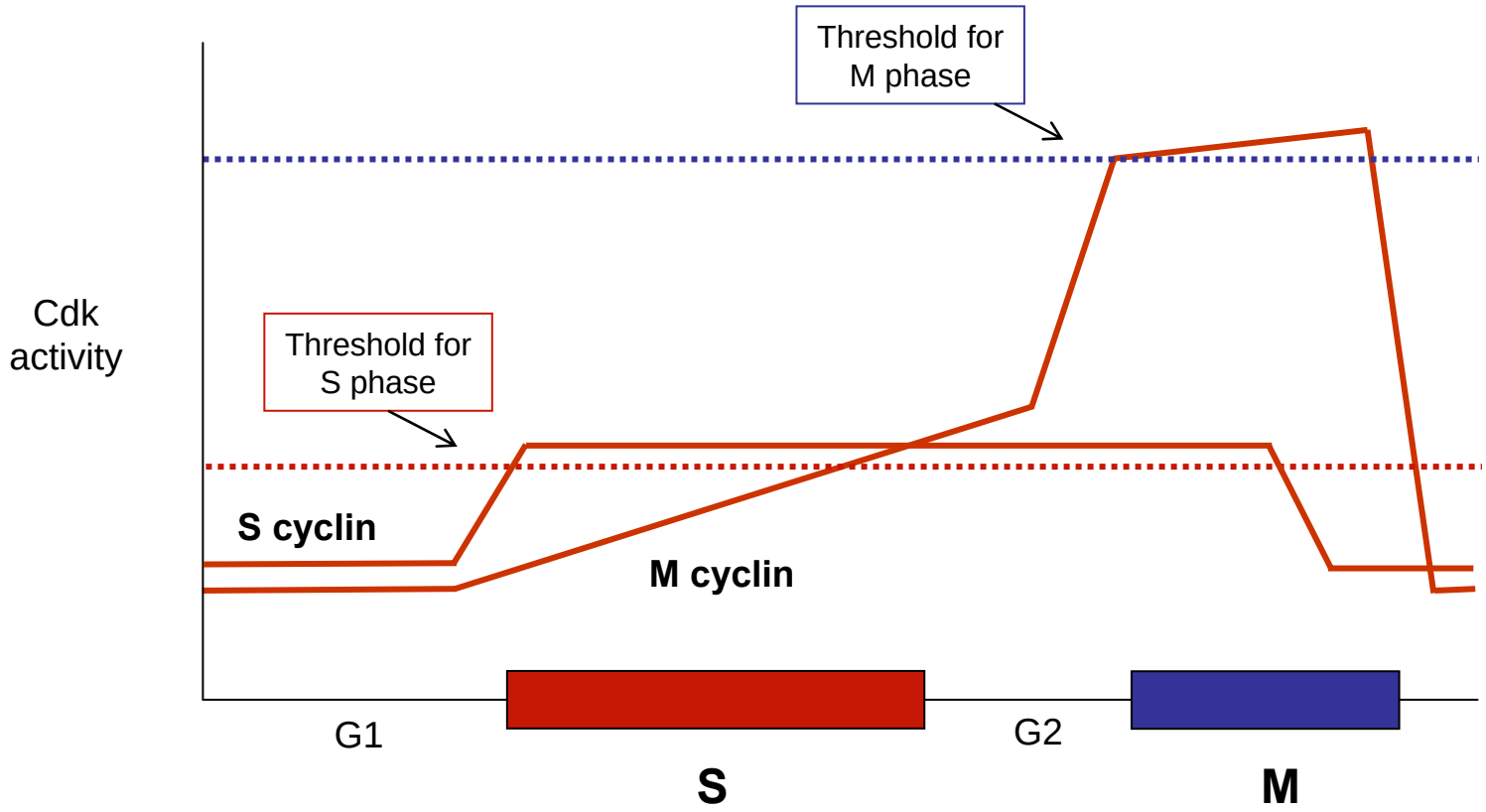
Ordered start of S-phase and M-phase in cell cycle



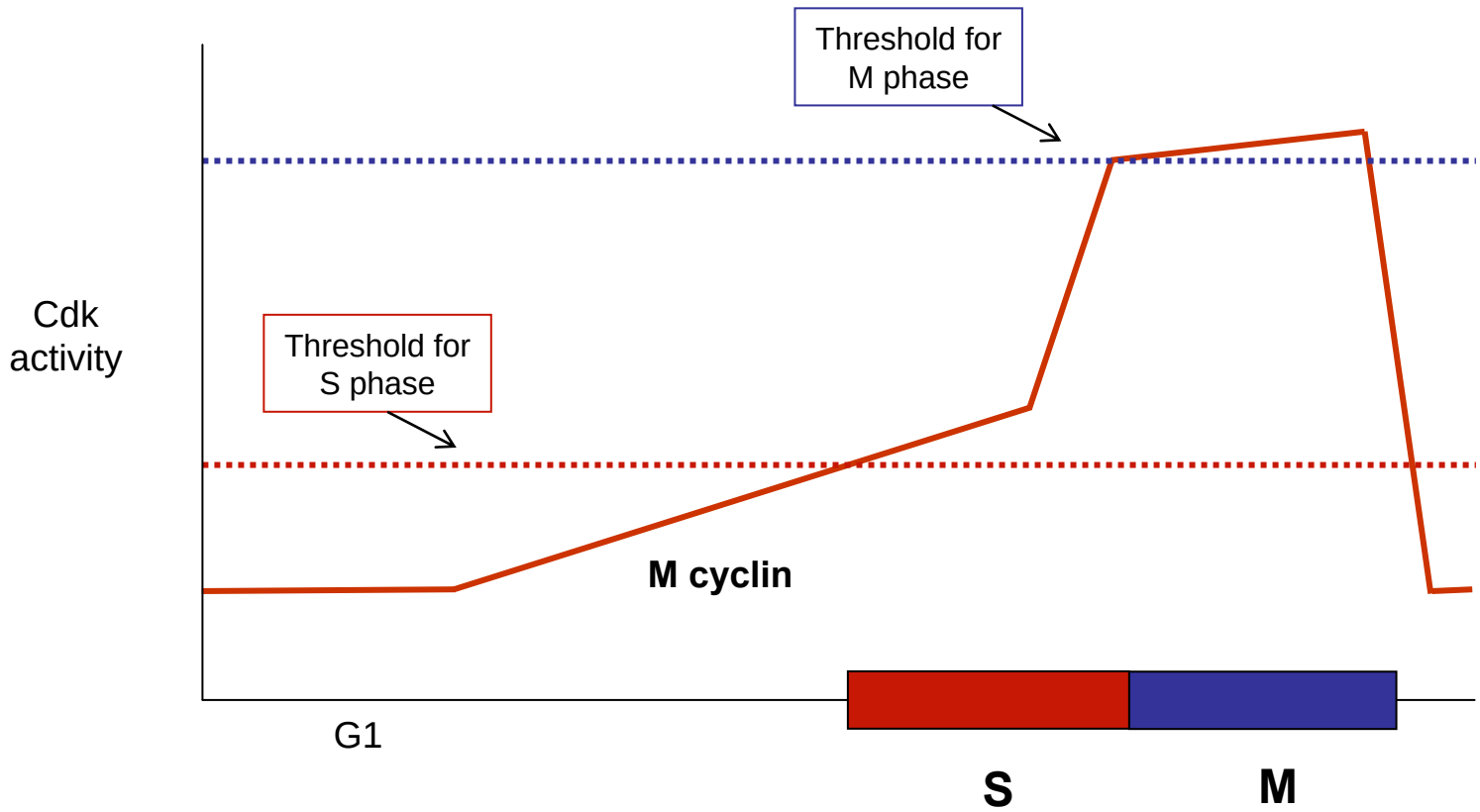
Cdks help put things in order



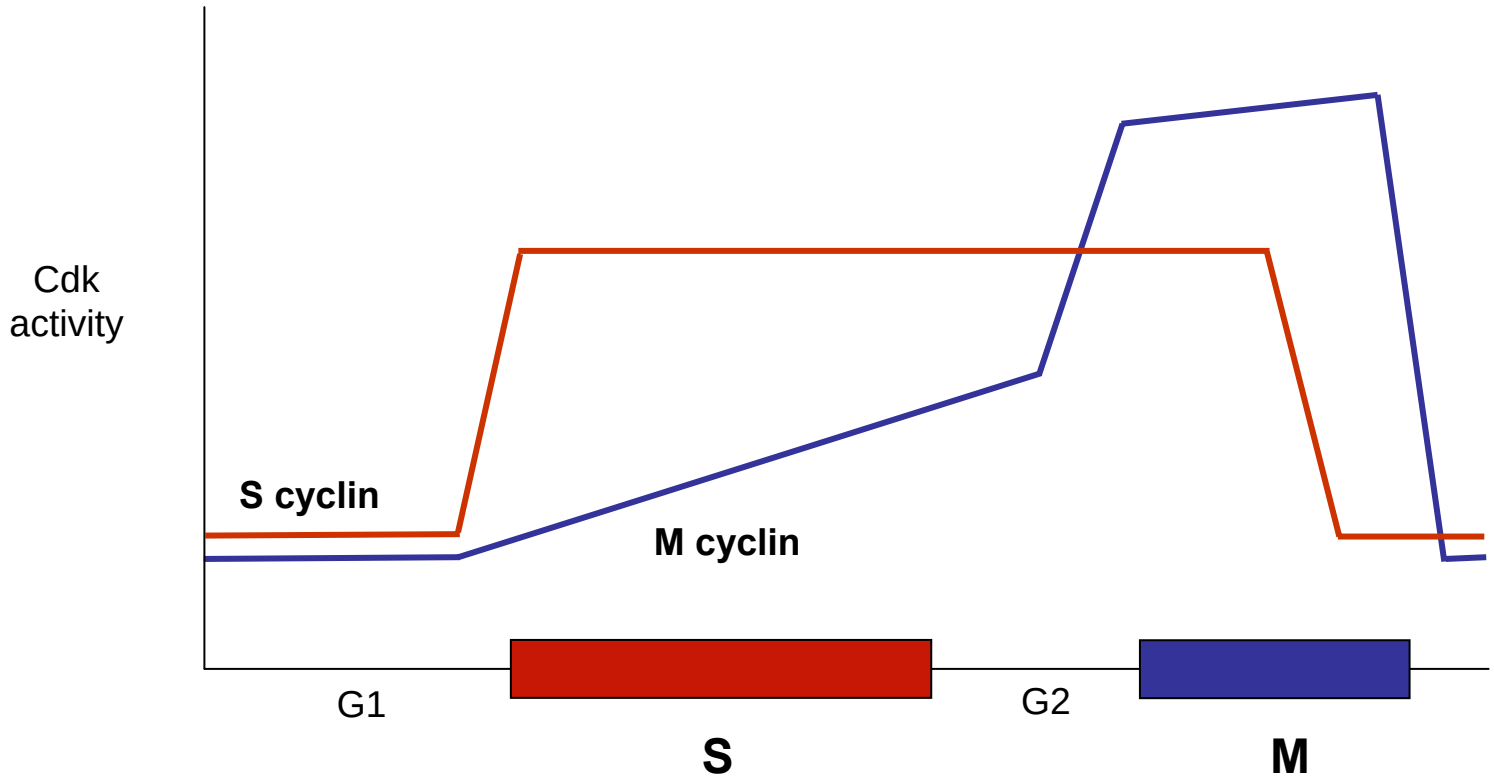
‘Quantitative model’ of cell-cycle control: cyclins are all the same



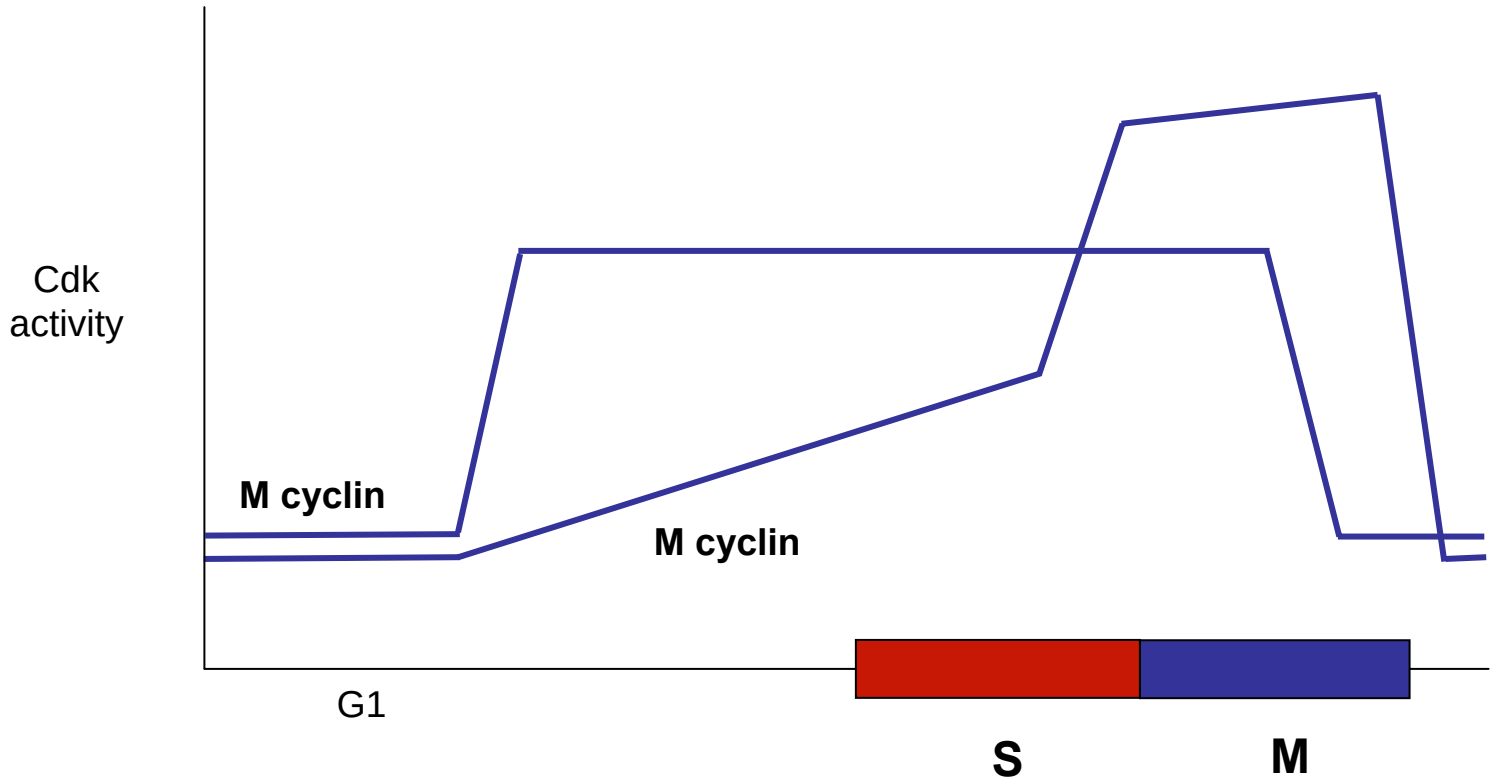
‘Quantitative model’ of cell-cycle control: evidence from fission yeast



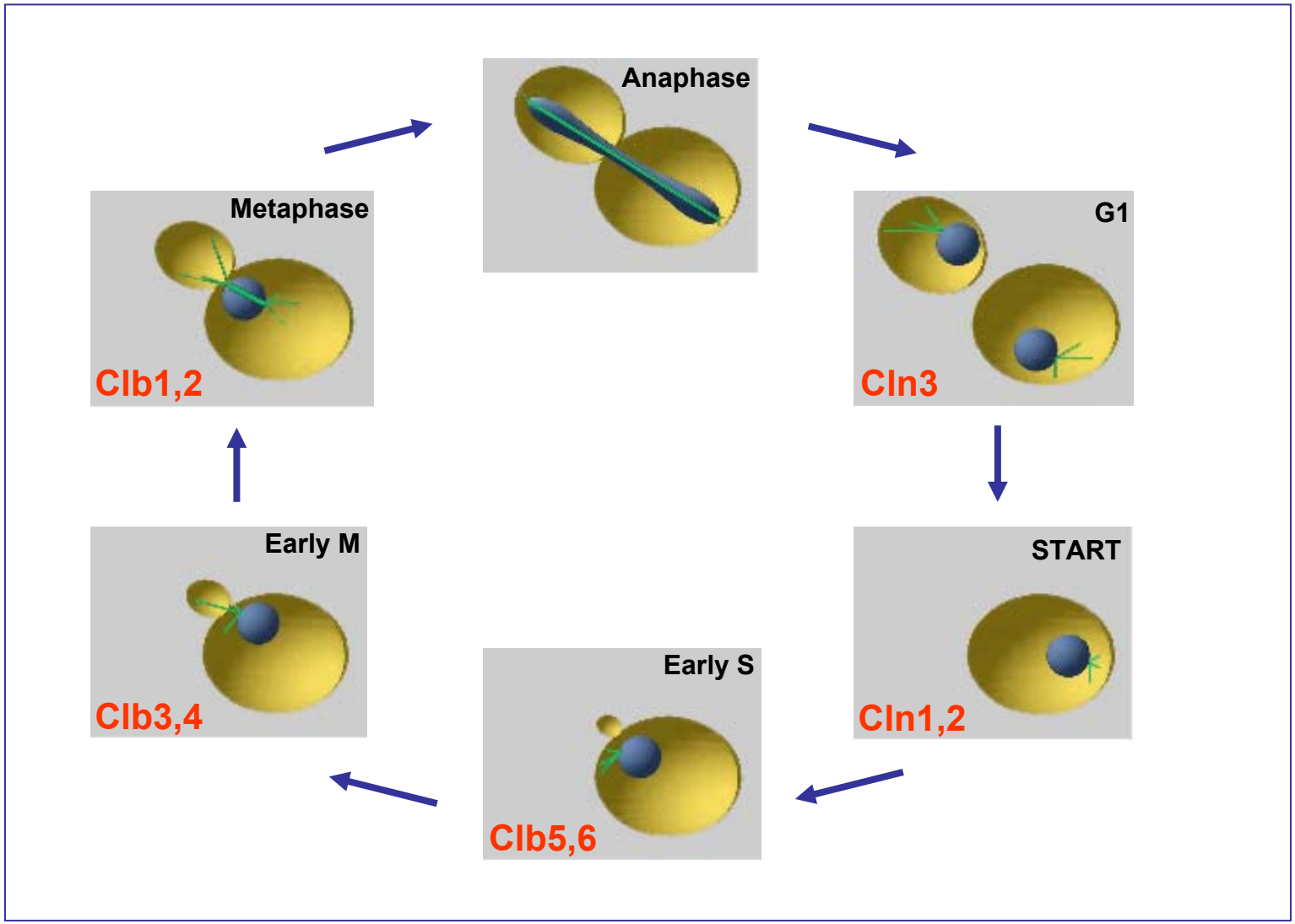
‘Qualitative model’: cyclins are intrinsically different



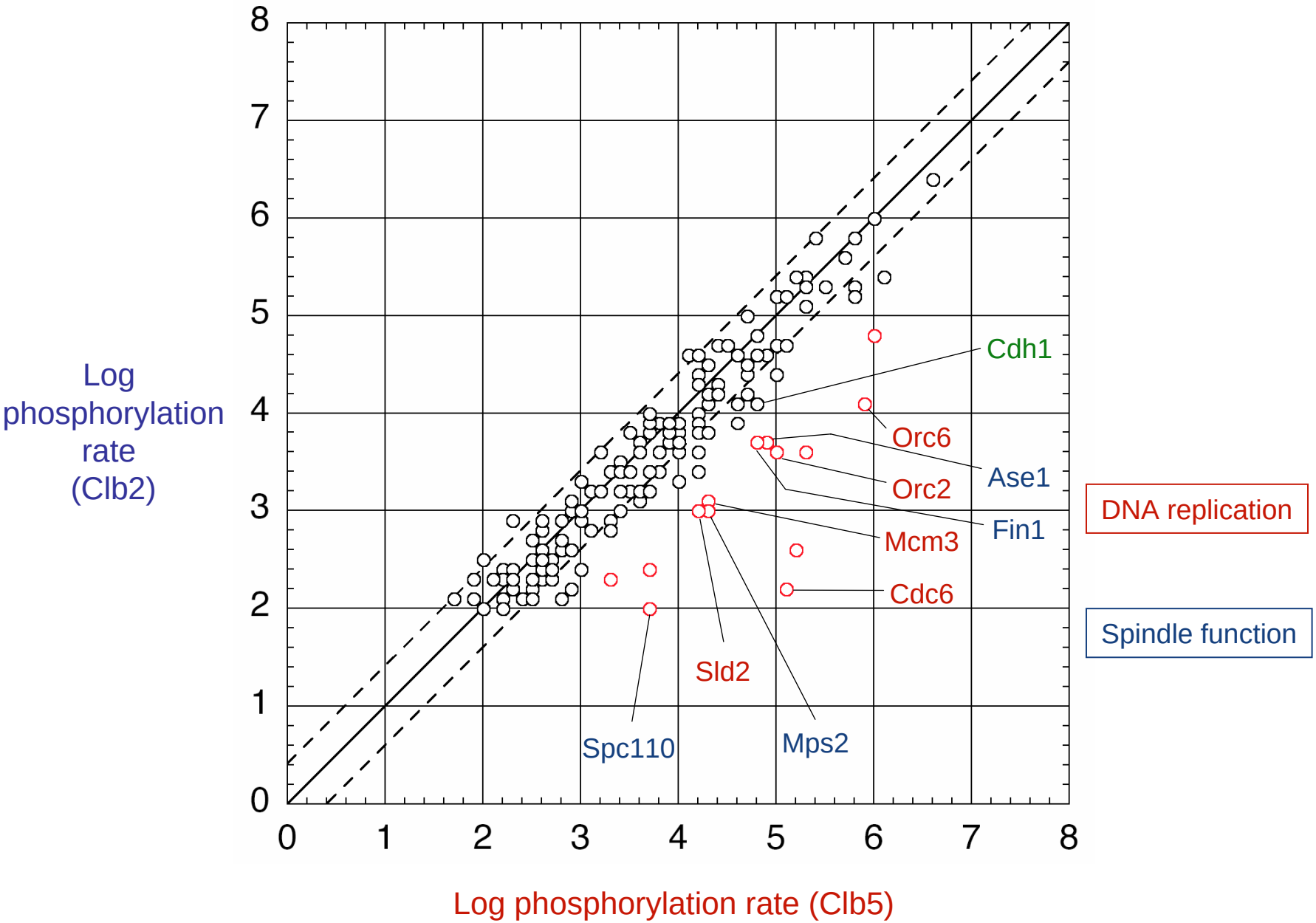
‘Qualitative model’: cyclins are intrinsically different: evidence from budding yeast



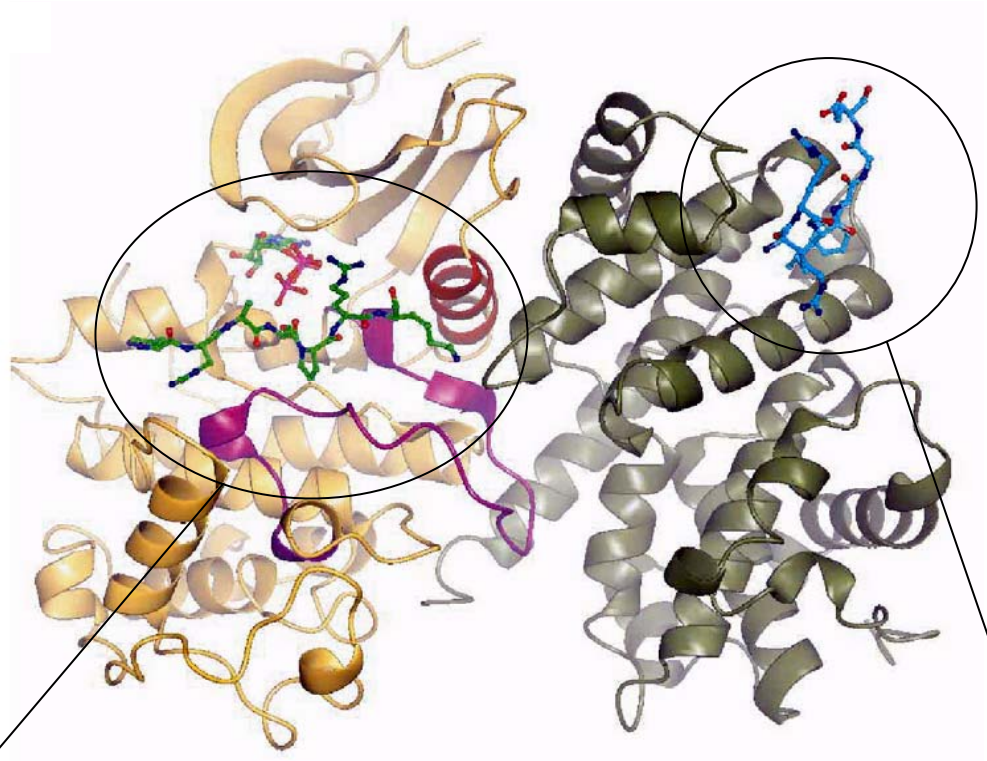
Cdk1/Cdc28 controls the budding yeast cell cycle



Phosphorylation of 150 Cdk1 substrates by Clb2 and Clb5



Substrate recognition by cyclin-Cdk complexes

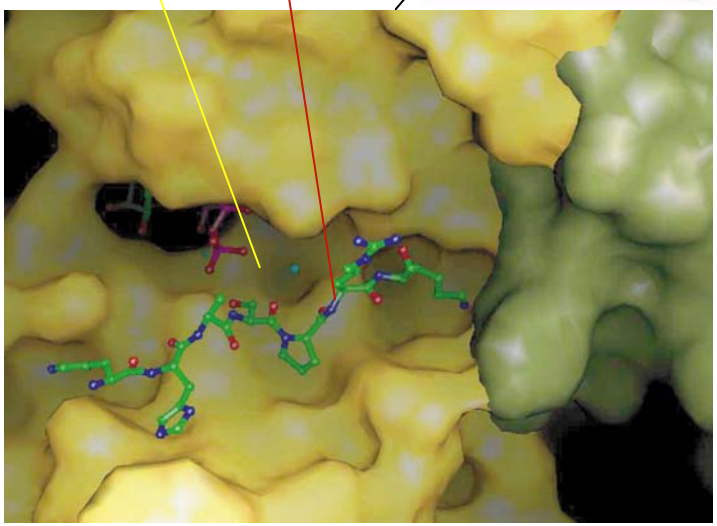


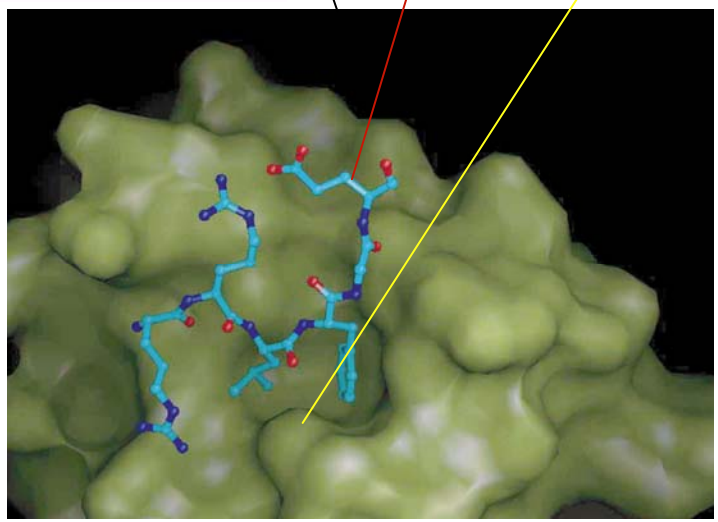
S*-P-x-K/R phosphorylation site on substrate

Active site of Cdk

'RXL' motif on substrate (p107, Cdc6)

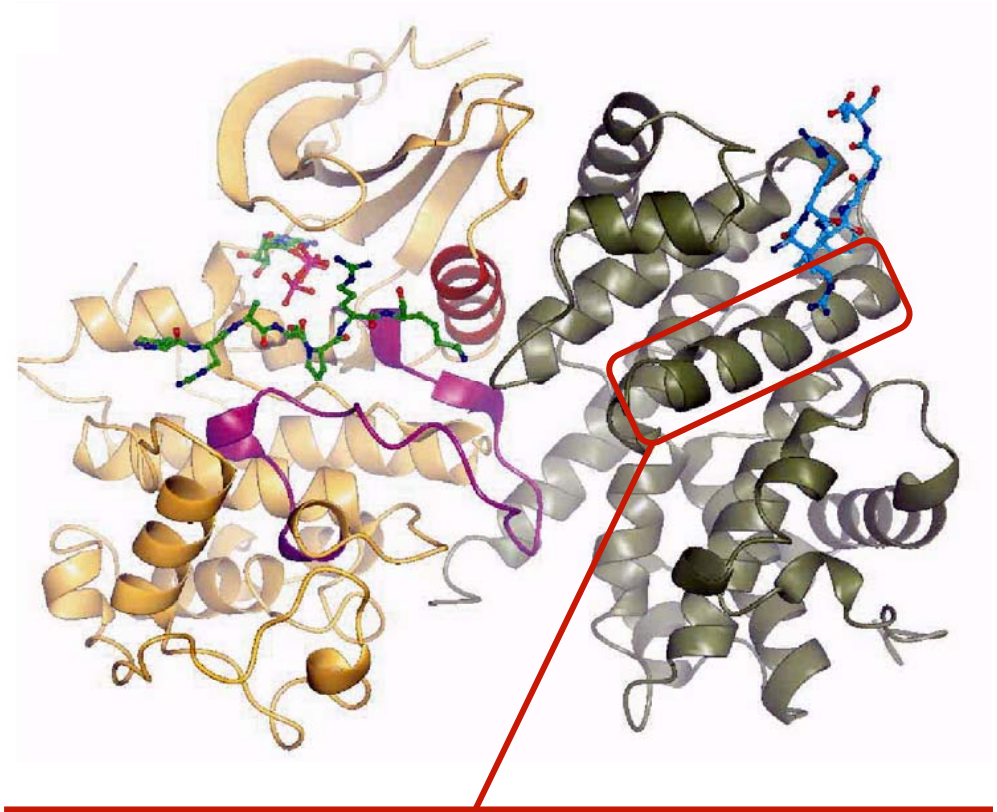
'hydrophobic patch' on cyclin





Brown et al. *Nat. Cell Biol.* **1**, 438 (1999)

Mutation of the hydrophobic patch in Clb2 and Clb5



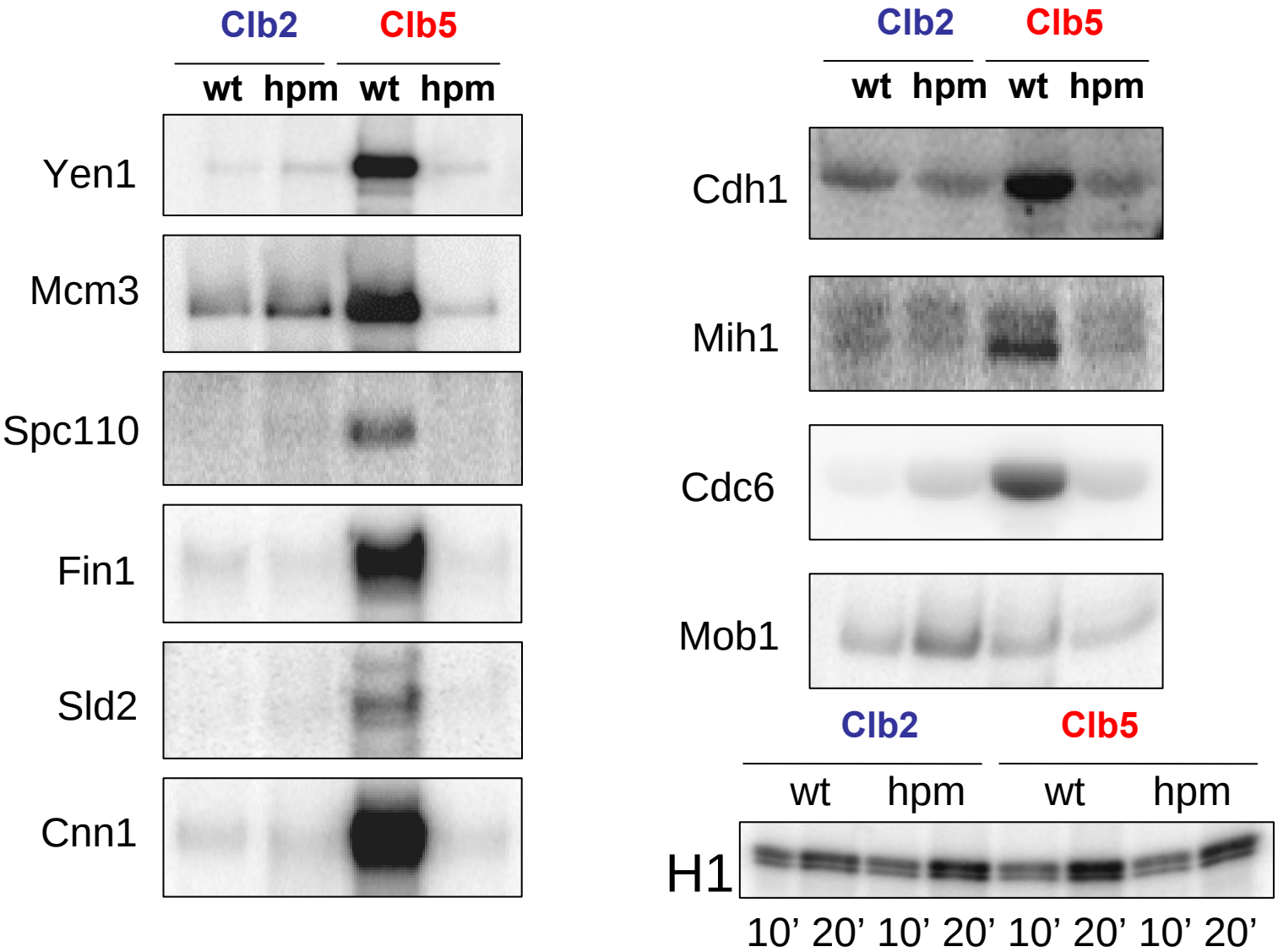
Clb2

wt	NIHQNRDILVNWLV**K**IHNKFGL
hpm mutant	NIHQ**A**RDI**A**VN**A**LVKIHNKFGL

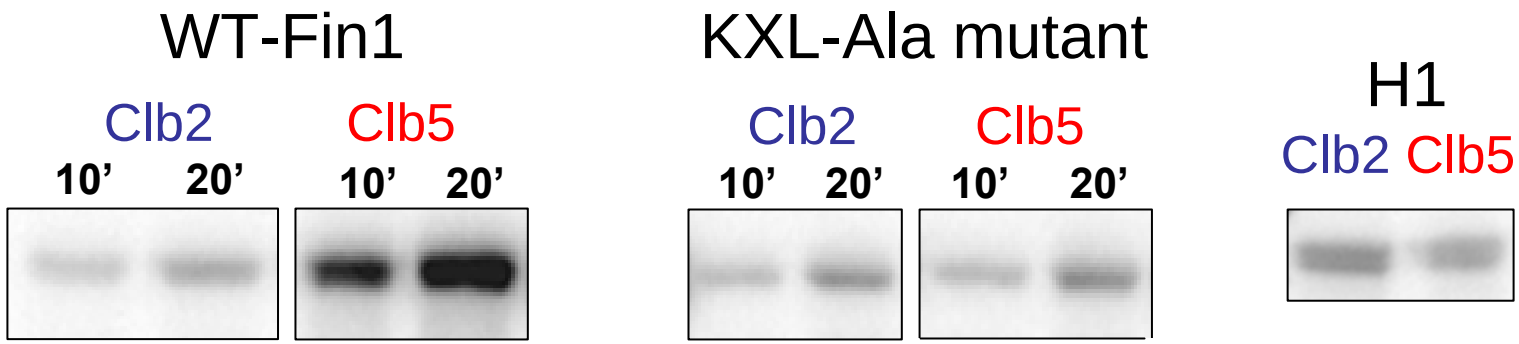
Clb5

wt	LRPSMRTILVDWLV**E**VHEKFQC
hpm mutant	LRPS**A**RTI**A**VD**A**LVEVHEKFQC

Hydrophobic patch is required for Clb5 specificity

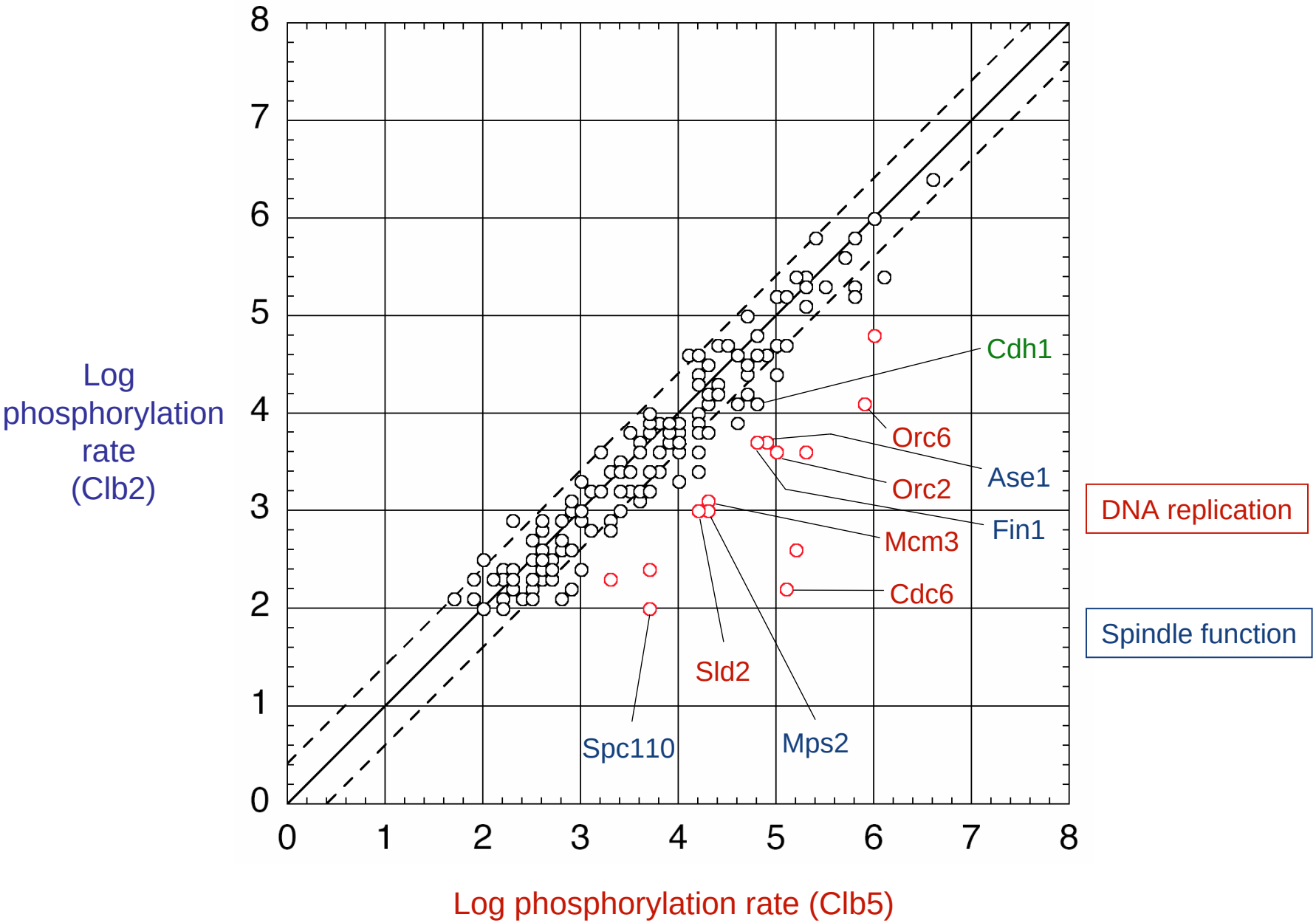


The critical ‘RXL’ motif in the Clb5-specific substrate Fin1

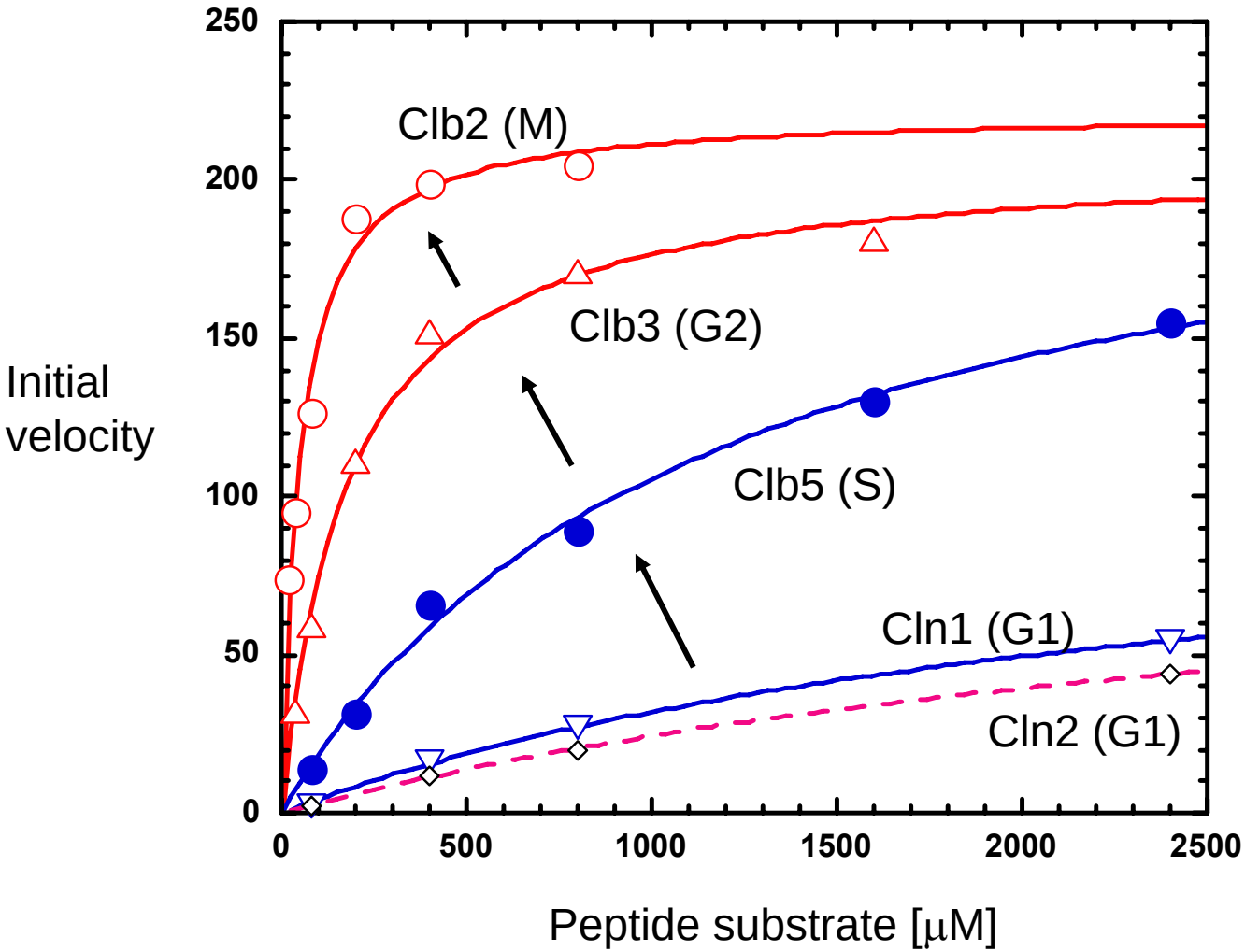


MSNKSNRRSLRDIGNTIGRNNIPSDKDNVFVRLSM**S**PLRT
TSQKEFLKPPMRI**S**PNKTDGMKHSIQV**T**PRRIM**S**PECLKG
YVSKETQSLDRPQFKNSNKNVKIQNSDHITNIIFPT**S**PTKLT
FSNENKIGGDGSLTRIRARFKNGLM**S**PERIQQQQQQQHILPS
DAKSNTDLCSNTELKDAPFENDLPRAKLKG**KNL**LVELKK
EEEDVGNGIESLTKSNTKLNSMLANEGKIHKASFQKSVKF
KLPDNIVTEETVELKEIKDLLLQMLRRQREIESRLSNIELQ
LTEIPKHK

Phosphorylation of 150 Cdk1 substrates by Clb2 and Clb5



Increasing kinase activity through the cycle

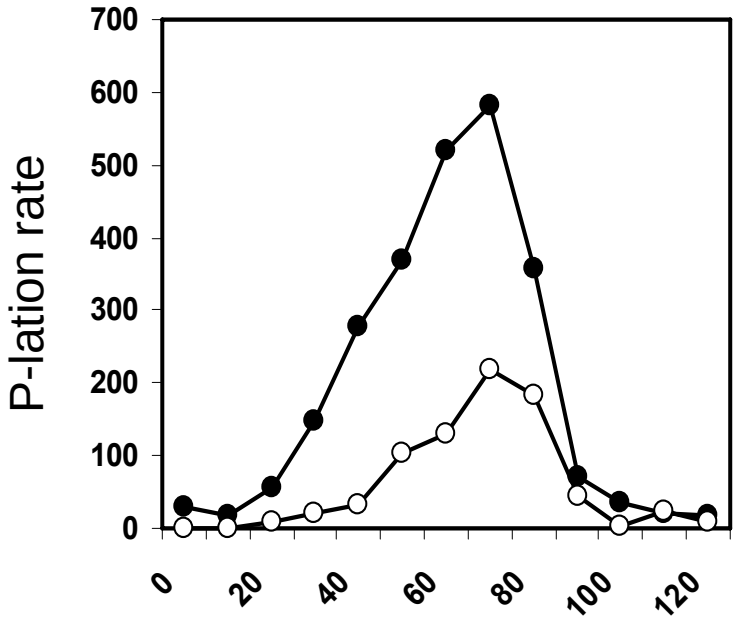


Increasing kinase activity through the cycle

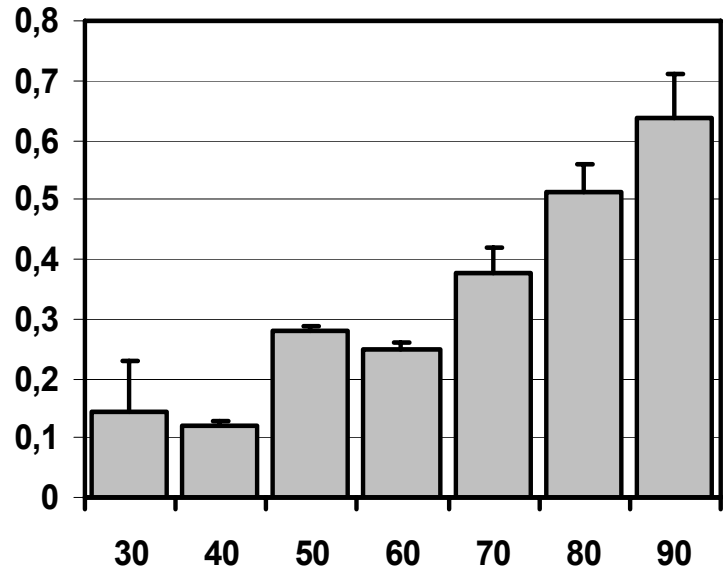
	Histone H1 peptide substrate		
	K_M (μM)	k_{cat} (s^{-1})	k_{cat}/K_M ($\mu M^{-1} \bullet s^{-1}$)
Clb2	46	31.5	0.68
Clb3	241	29.3	0.12
Clb5	521	22.0	0.042
Cln2	1145	1.9	0.0016

Gradually changing kinetic properties of Cdk1 during the cell-cycle

Cdk1-cyclin complexes from Cks1-bead pulldowns



Relative P-lation rate: 0.05mM/2mM

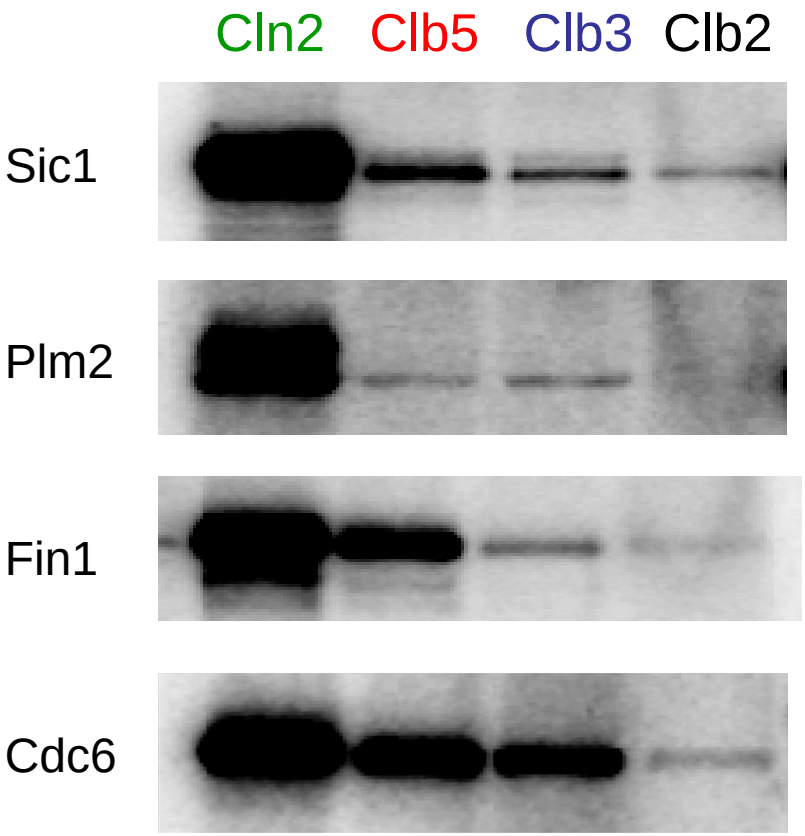


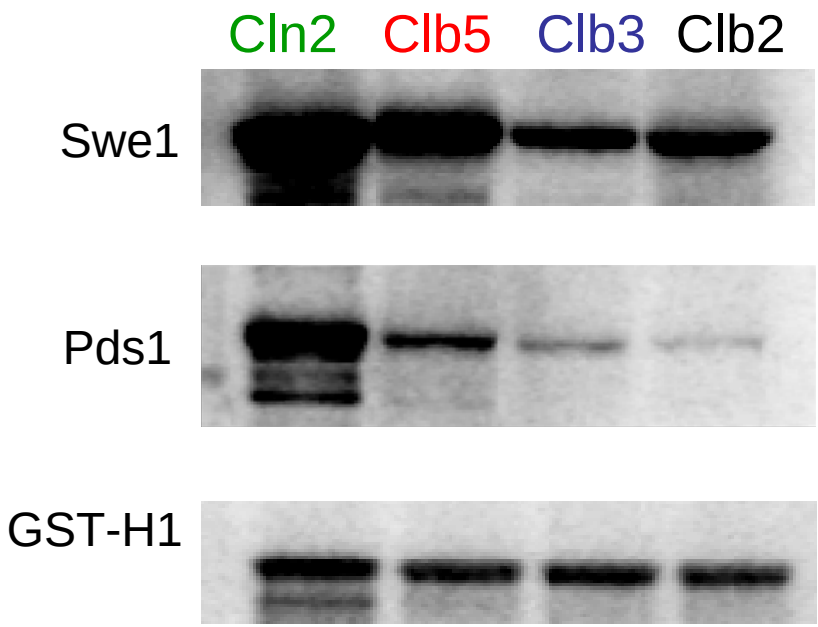
Minutes from alfa-factor release

● - 2 mM peptide (~Vmax)

○ - 0.05 mM peptide (<Km)

Cln2-specificity with protein substrates containing multiple phosphorylation sites





Multisite phosphorylation of a CDK inhibitor sets a threshold for the onset of DNA replication

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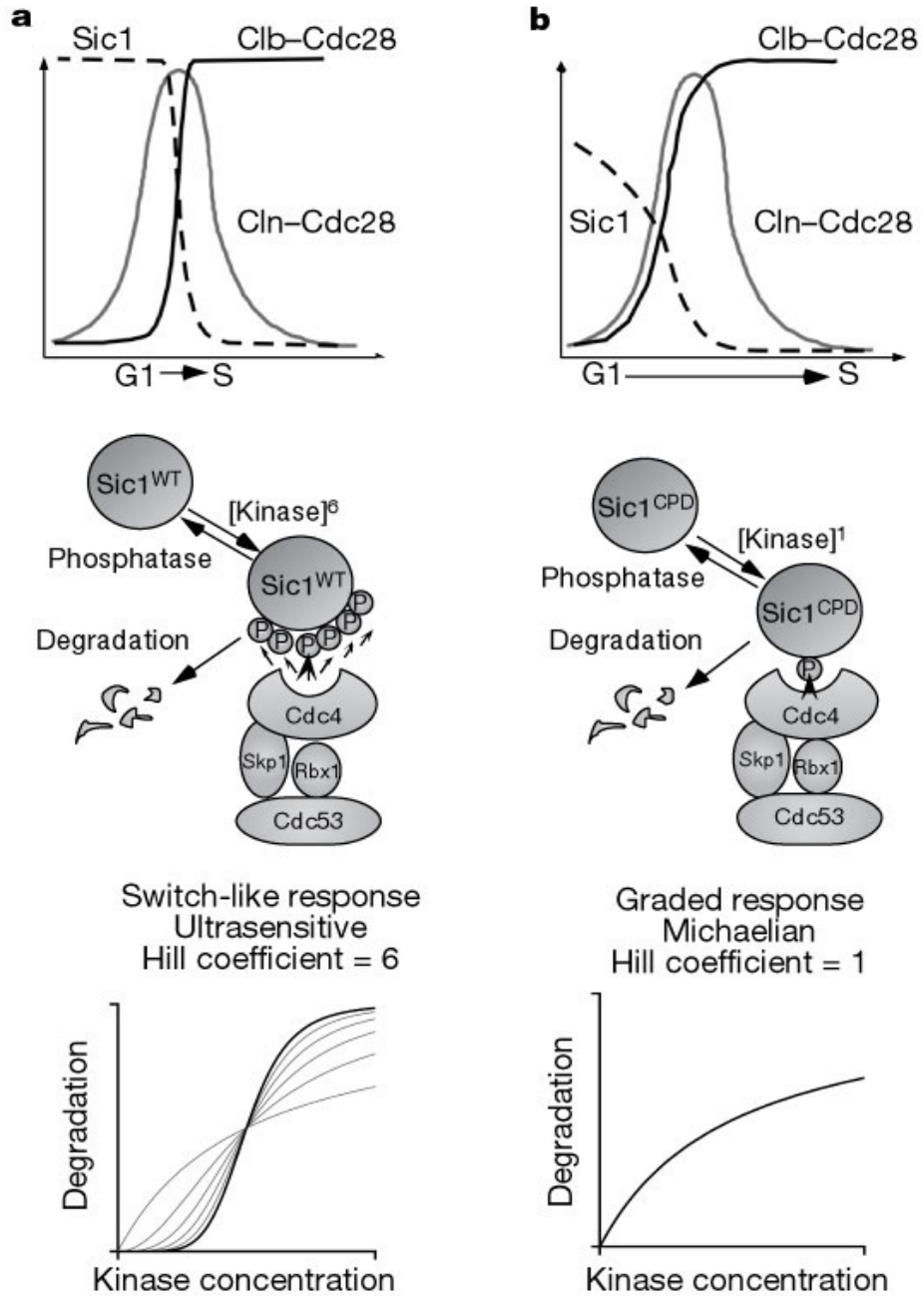
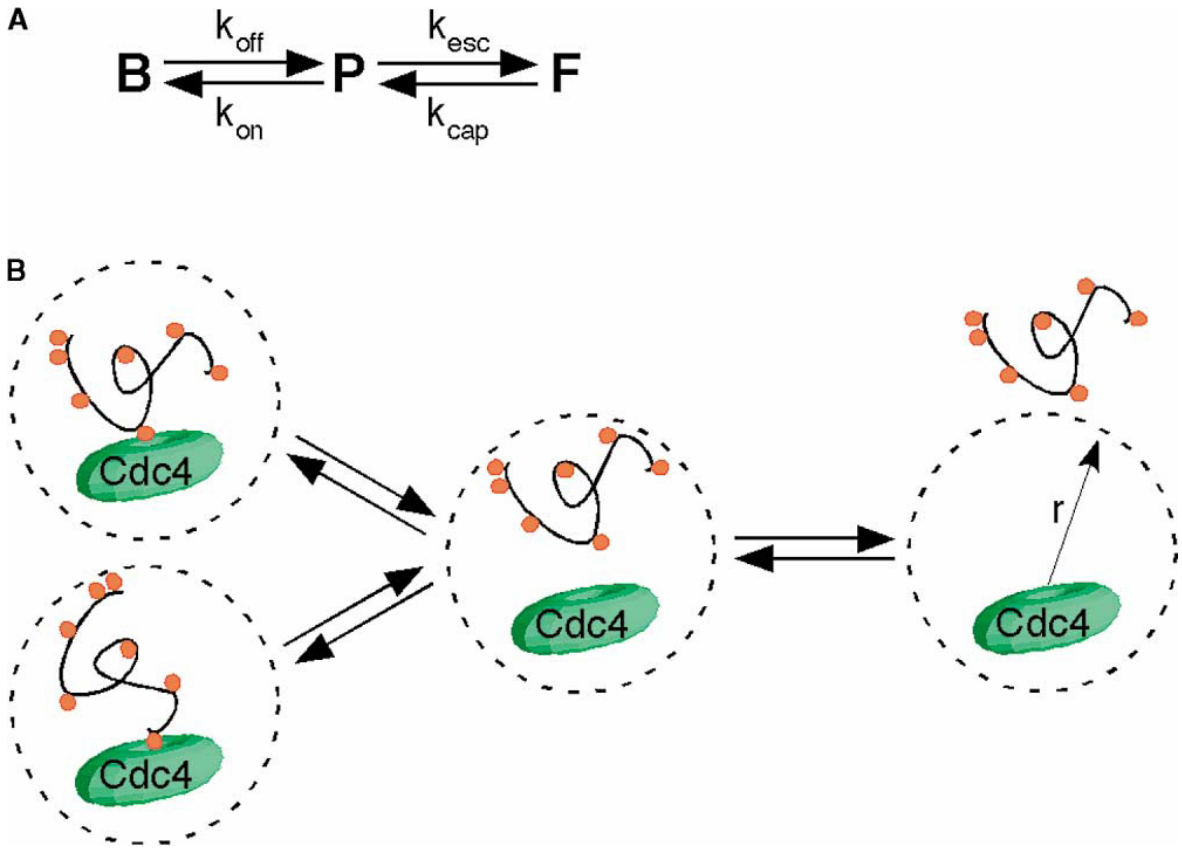
† These authors contributed equally to this work

SCF ubiquitin ligases target phosphorylated substrates for ubiquitin-dependent proteolysis by means of adapter subunits called F-box proteins. The F-box protein Cdc4 captures phosphorylated forms of the cyclin-dependent kinase inhibitor Sic1 for ubiquitination in late G1 phase, an event necessary for the onset of DNA replication. The WD40 repeat domain of Cdc4 binds with high affinity to a consensus phosphopeptide motif (the Cdc4 phospho-degron, CPD), yet Sic1 itself has many sub-optimal CPD motifs that act in concert to mediate Cdc4 binding. The weak CPD sites in Sic1 establish a phosphorylation threshold that delays degradation *in vivo*, and thereby establishes a minimal G1 phase period needed to ensure proper DNA replication. Multisite phosphorylation may be a more general mechanism to set thresholds in regulated protein–protein interactions.

Numerous regulatory proteins are targeted for degradation in a precisely programmed manner through the covalent conjugation of ubiquitin, which is transferred along a cascade of E1, E2 and E3 enzymes to the substrate[1]. Reiterative transfer of ubiquitin generates polyubiquitinated species that are recognized and rapidly degraded by the 26S proteasome. E3 enzymes, or ubiquitin ligases, catalyse the terminal step in ubiquitin transfer, and as such are the crucial determinants of substrate specificity. Substrate recognition depends on often ill-defined sequence elements, referred to as degrons, that are the binding sites for cognate E3 enzymes[1,2]. The E3–substrate interaction can be regulated at several levels. In some instances, limiting cofactors determine E3 activity, as in the case of the anaphase promoting complex/cyclosome (APC/C), which targets mitotic cyclins and other proteins for degradation during mitosis[3].

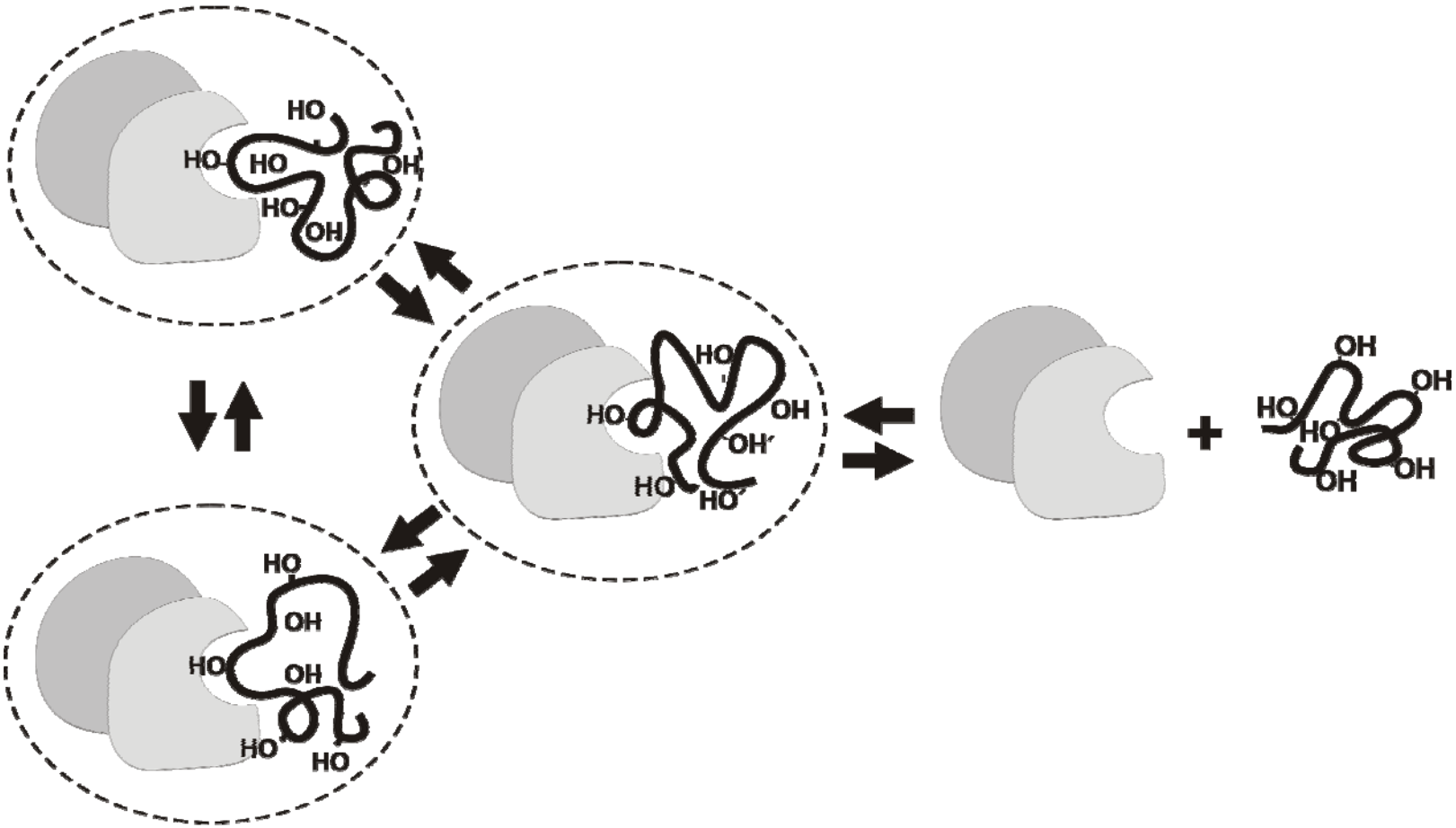
degradation[6,7]. Phospho-Sic1 is specifically recognized by the F-box protein Cdc4, which recruits Sic1 for ubiquitination by the Cdc34–SCF complex[4,5,8]. Overexpression of stabilized forms of Sic1 that lack Cdc28 phosphorylation sites cause an arrest at the G1 phase[9], whereas deletion of *SIC1* causes premature DNA replication and rampant genome instability[10]. Cdc4 recruits several other substrates to the SCF core complex in a phosphorylation-dependent manner, including the Cln–Cdc28 inhibitor/cytoskeletal scaffold protein Far1, the replication protein Cdc6 and the transcription factor Gcn4 (ref. 3). In the mammalian cell cycle, SCF complexes target phosphorylated forms of cyclin E1 and the CDK inhibitor $p27^{Kip1}$ (refs 11, 12). The important role of SCF pathways is shown by the G1 phase arrest caused by non-phosphorylatable forms of $p27^{Kip1}$, and by the genome instability caused by expression of


a
Sic1
Clb–Cdc28
Cln–Cdc28
G1
S
b
Clb–Cdc28
Sic1
Cln–Cdc28
G1
S
Sic1WT
[Kinase]6
Phosphatase
Sic1WT
Degradation
P
Cdc4
Skp1
Rbx1
Cdc53
Sic1CPD
[Kinase]1
Phosphatase
Sic1CPD
Degradation
P
Cdc4
Skp1
Rbx1
Cdc53
Switch-like response
Ultrasensitive
Hill coefficient = 6
Degradation
Kinase concentration
Graded response
Michaelian
Hill coefficient = 1
Degradation
Kinase concentration



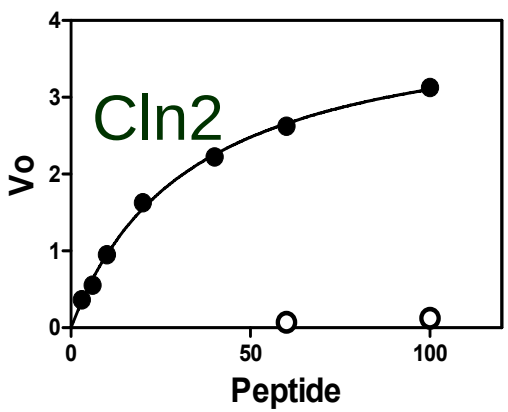
Mike Tyers and collegues, Current Biol. 13, 1669-1678, 2003

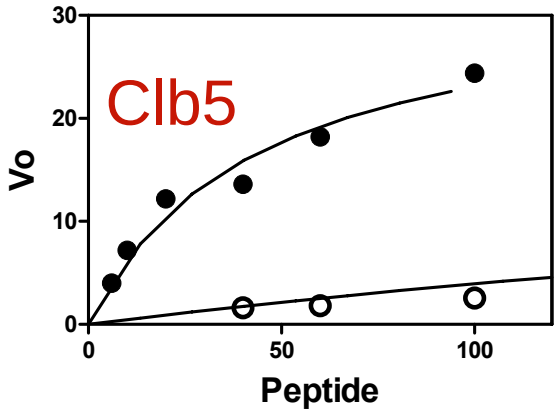
Multisite substrates can be highly specific for Cln2-Cdk1 due to the “dynamic polyvalent interaction”

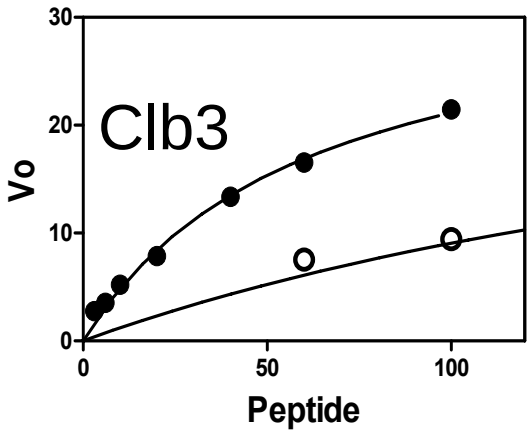


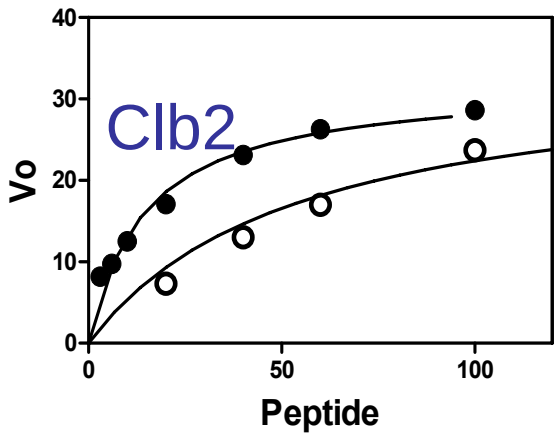
Peptide with multiple Cdk-sites shows high specificity for Cln2-Cdk1

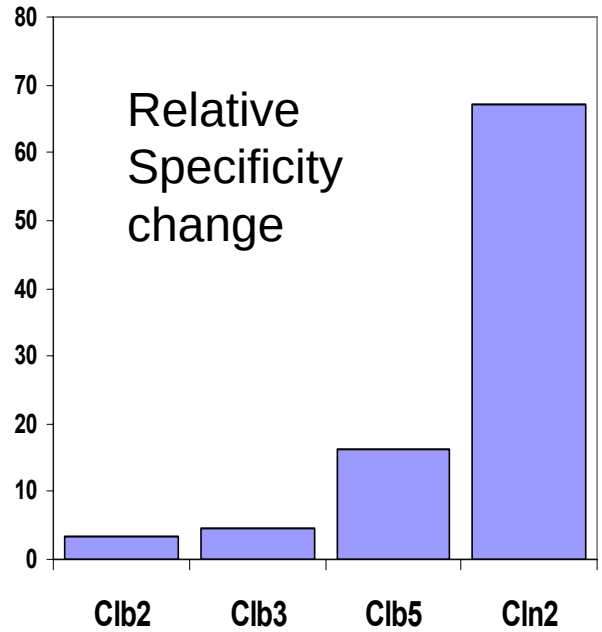
- PKTPKKAPKTPKKAPKTPKKAPKTPKKAPKTPKKA
- PKTPKKAKKL



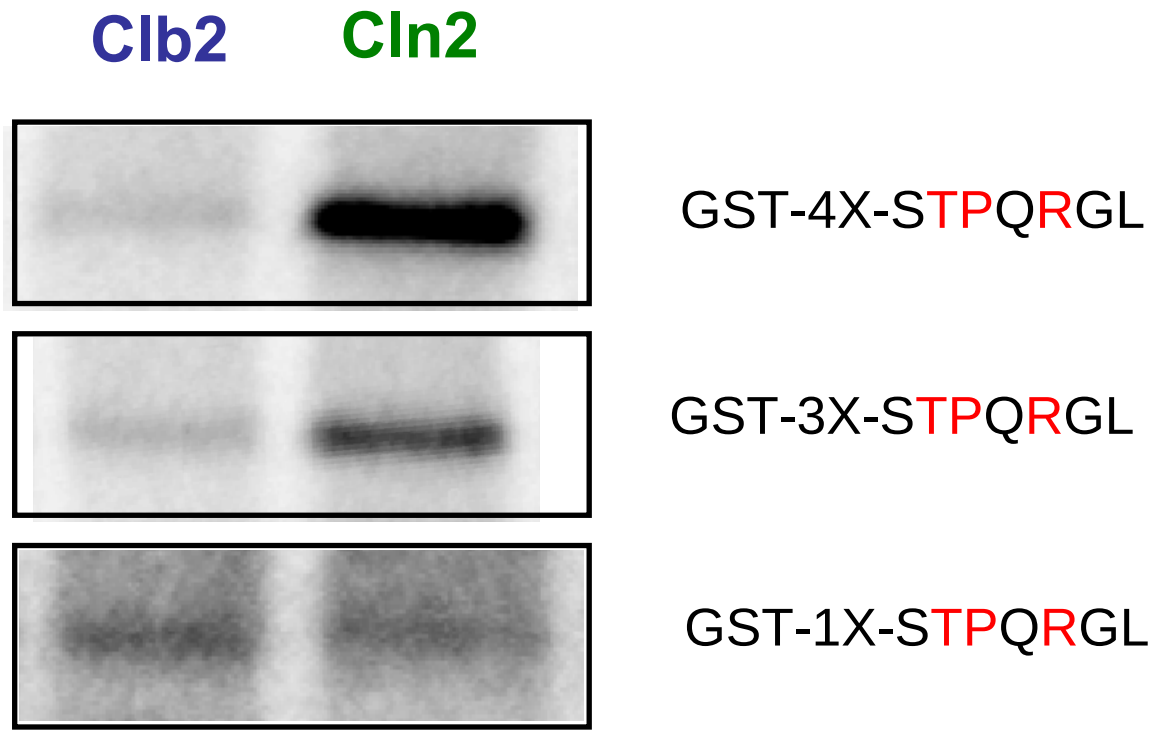








Cln2-specificity with artificial polyvalent substrates

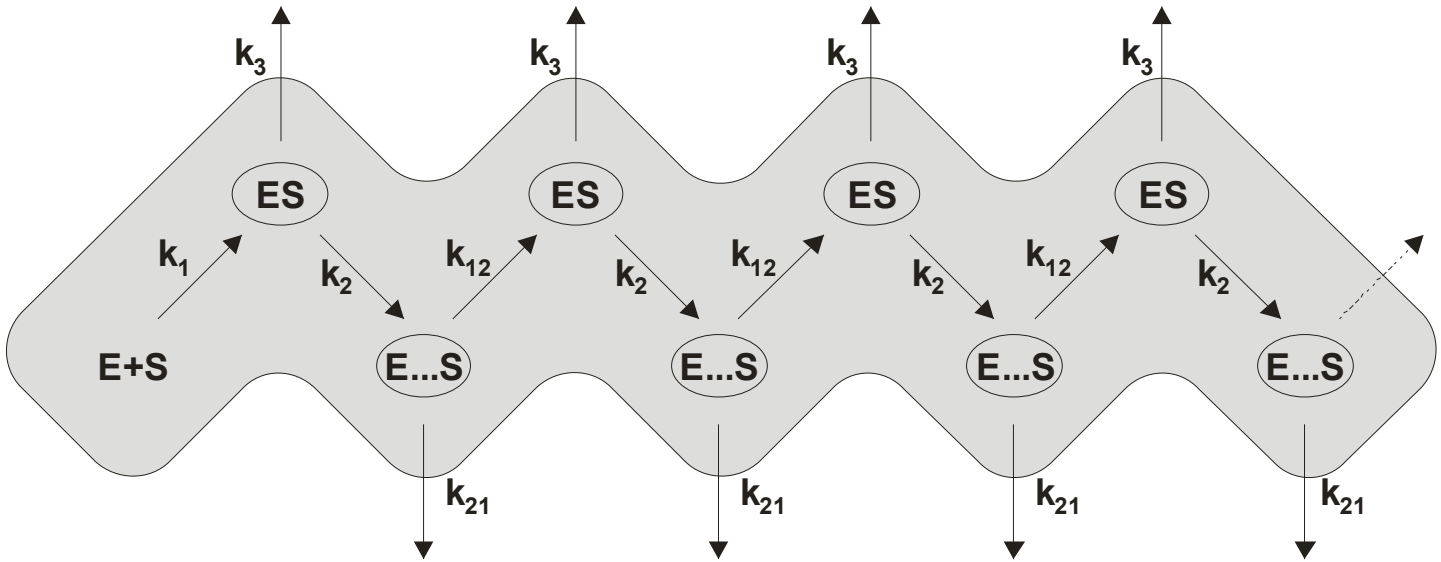


$$E \cdot ADP \underset{k_2}{\overset{k_1}{\rightleftarrows}} E \cdot ATP \cdot S \xrightarrow{k_3} E \cdot ADP \cdot P \xrightarrow{k_4} E + P + ADP$$

$$k_{cat} = \frac{k_3 k_4}{k_3 + k_4}$$

$$K_M = \frac{k_2 + k_3}{k_1} \times \frac{k_4}{k_3 + k_4}$$

$$k_{cat} / K_M = \frac{k_1 k_3}{k_2 + k_3}$$

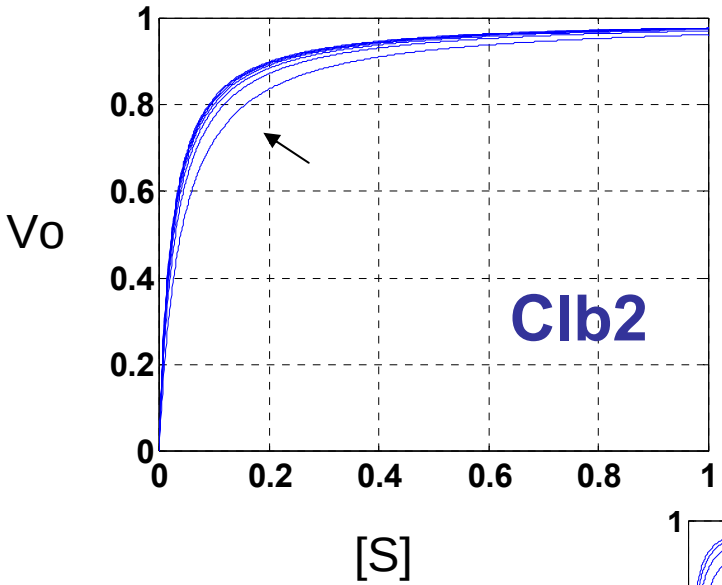


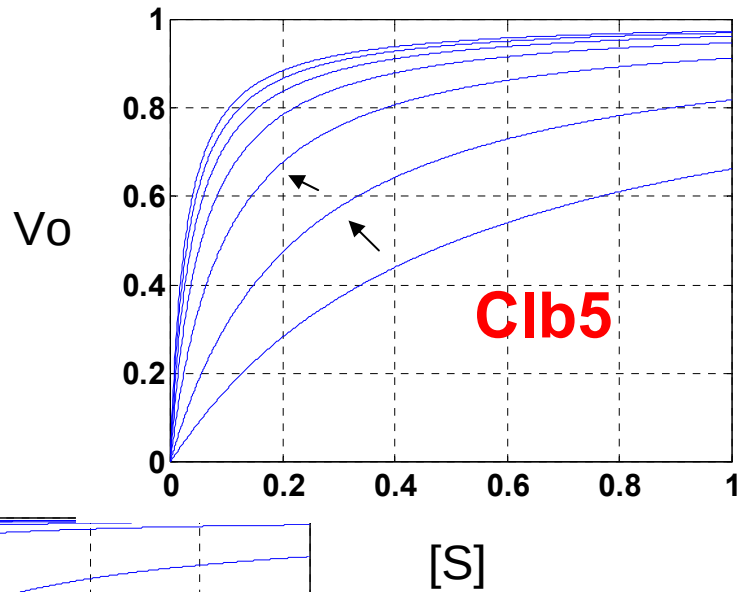
$$k_{cat} / K_M = \frac{k_1 k_3}{k_2 + k_3} \qquad k_{cat} / K_M = \frac{k_1 k_{3TOTAL}}{k_{2TOTAL} + k_{3TOTAL}}$$

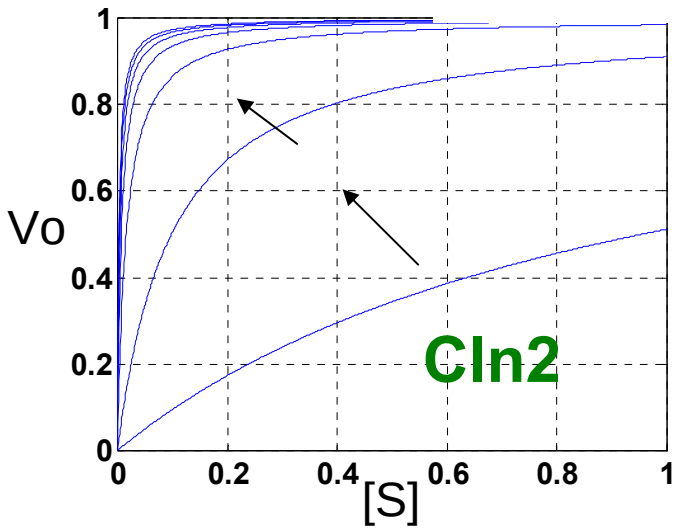
$$k_{cat} / K_M = k_1 (1 - ac(1 + ab + a^2 b^2 + a^3 b^3 + \ldots + a^N b^N))$$

$$a = \frac{k_2}{k_2 + k_3} \qquad b = \frac{k_{12}}{k_{12} + k_{21}} \qquad c = \frac{k_{21}}{k_{12} + k_{21}}$$

Relative specificity of polyvalent substrates is highly sensitive to escape-rebind ratio

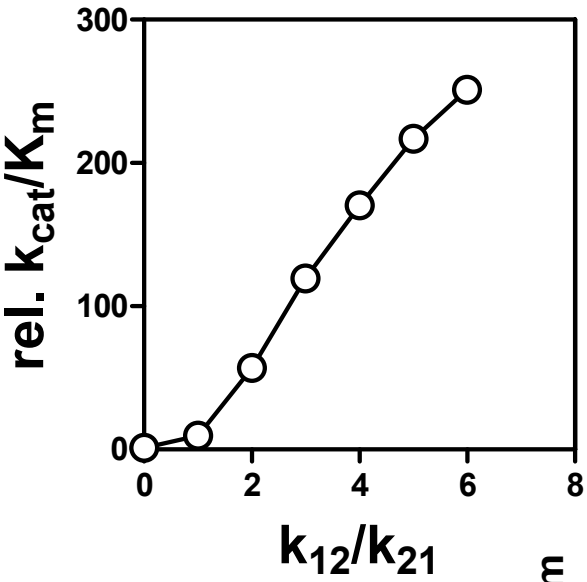


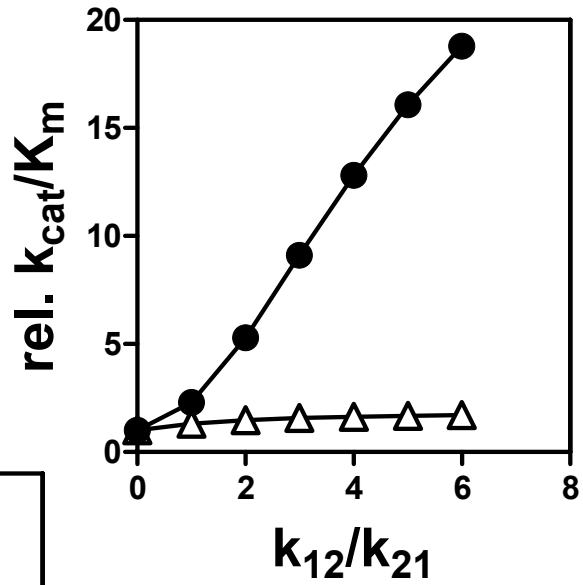


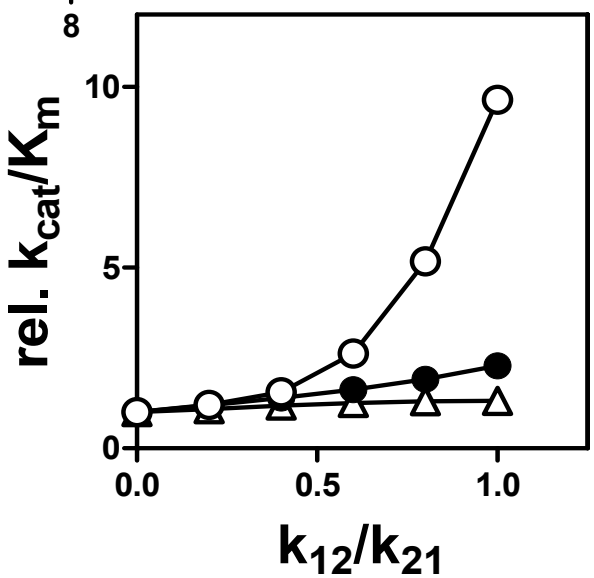


Increasing k_{12}/k_{21}

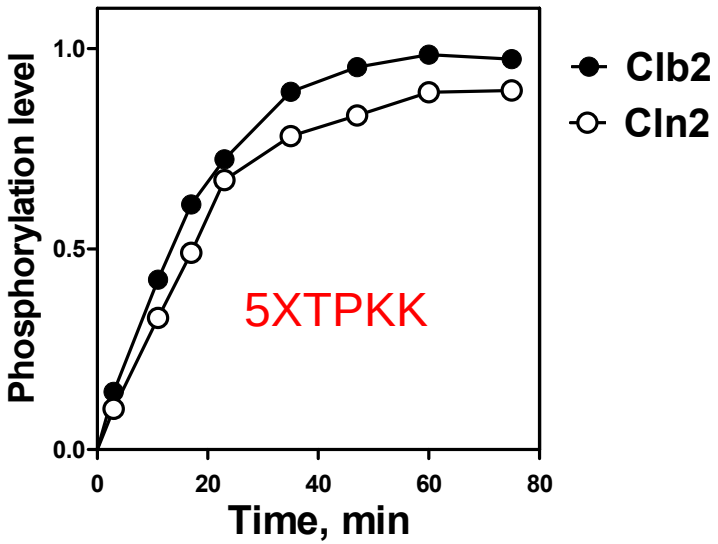
Relative specificity of polyvalent substrates is highly sensitive to escape-rebind ratio

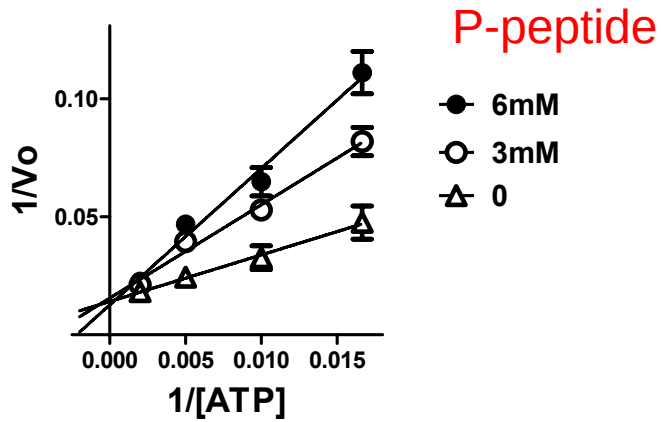


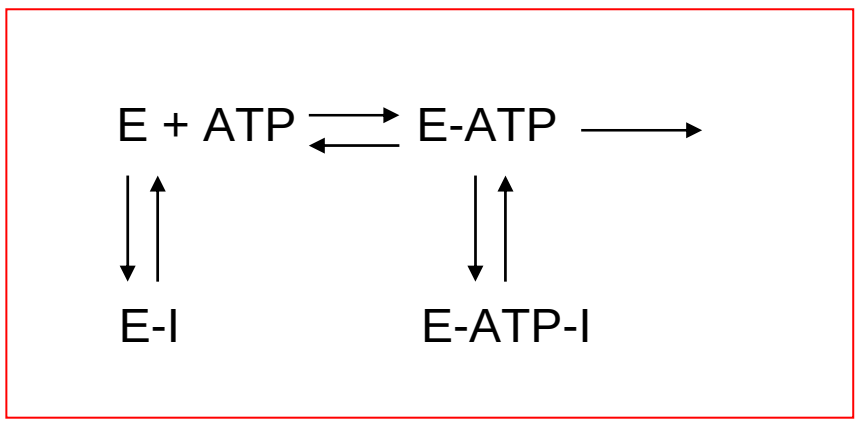


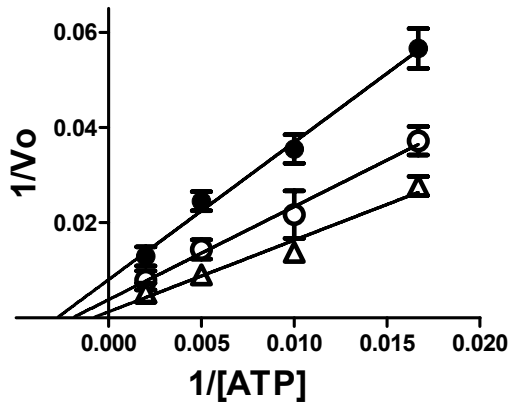


Phosphorylated sites may participate in polyvalent interaction between Cln2-Cdk1 and substrates

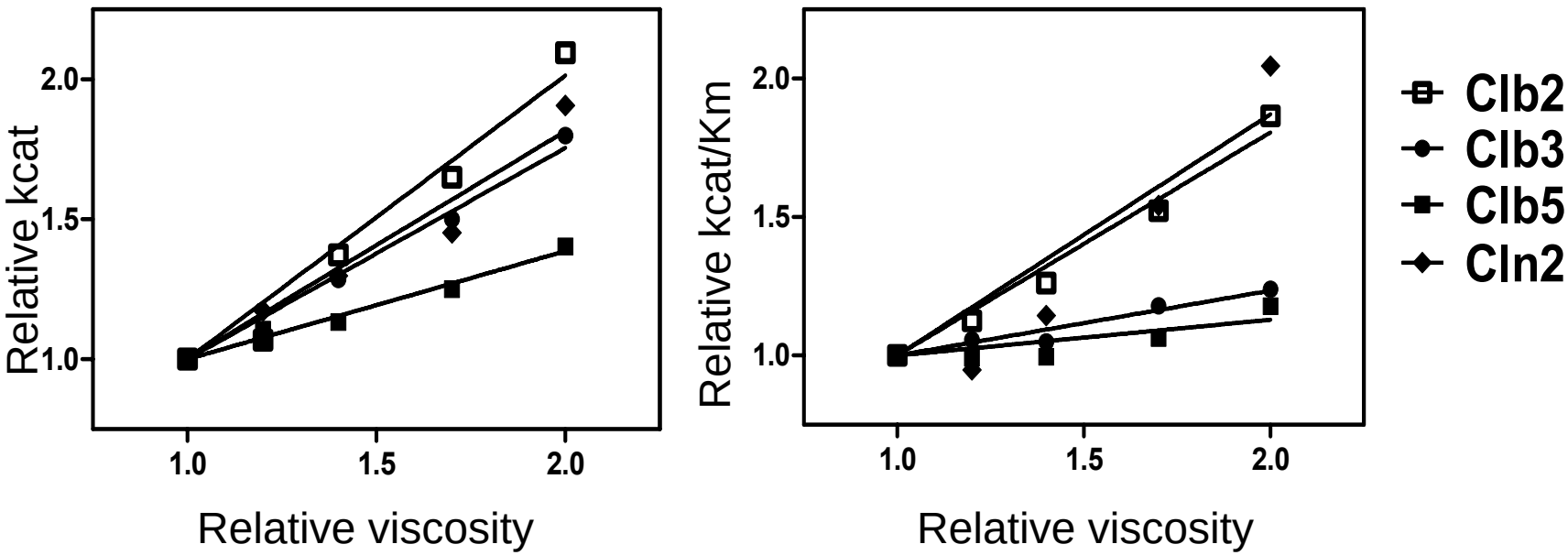








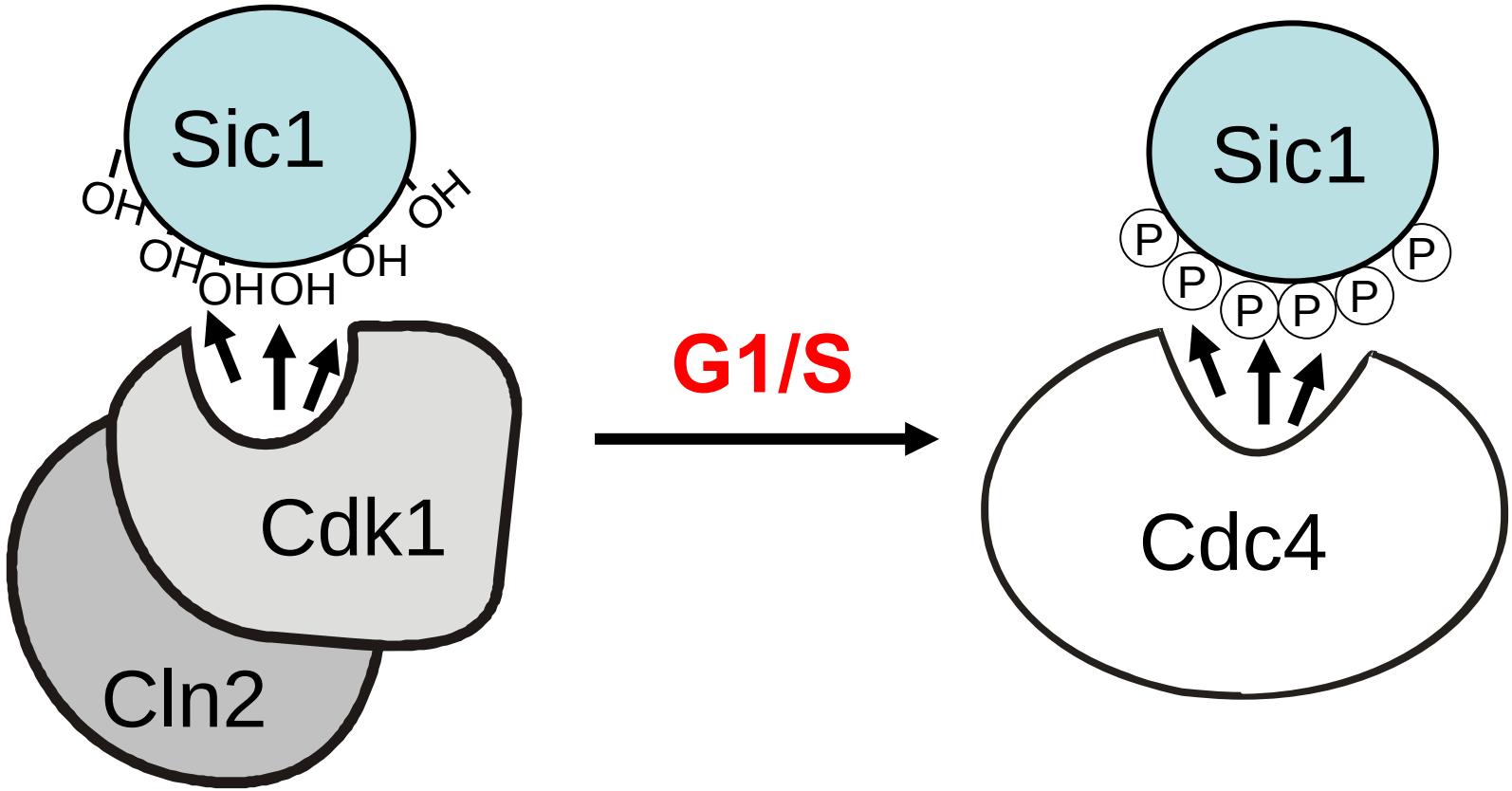
Variation of solvent viscosity



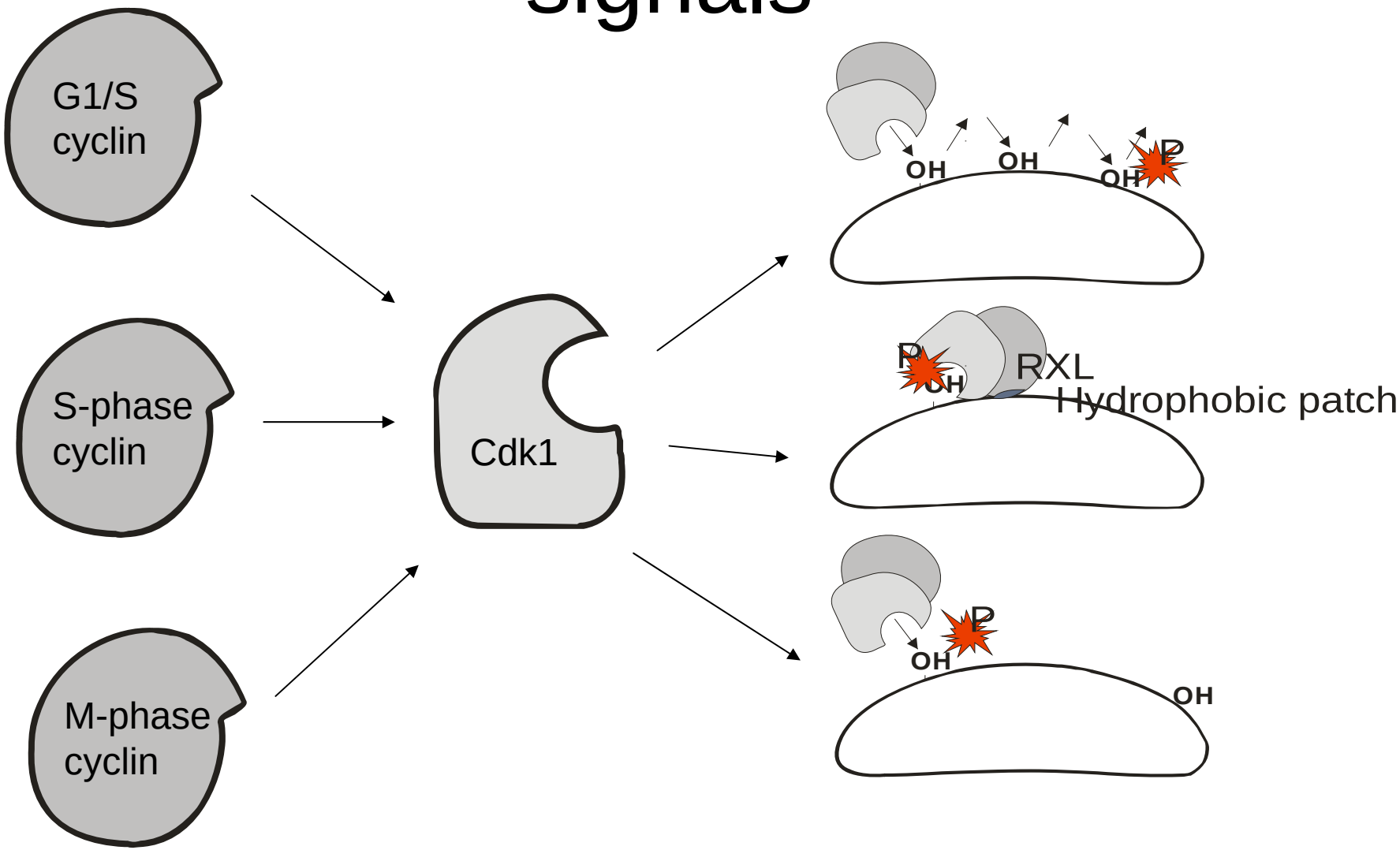
SLOPE: kcat/kcatη = k3/(k3+k4)

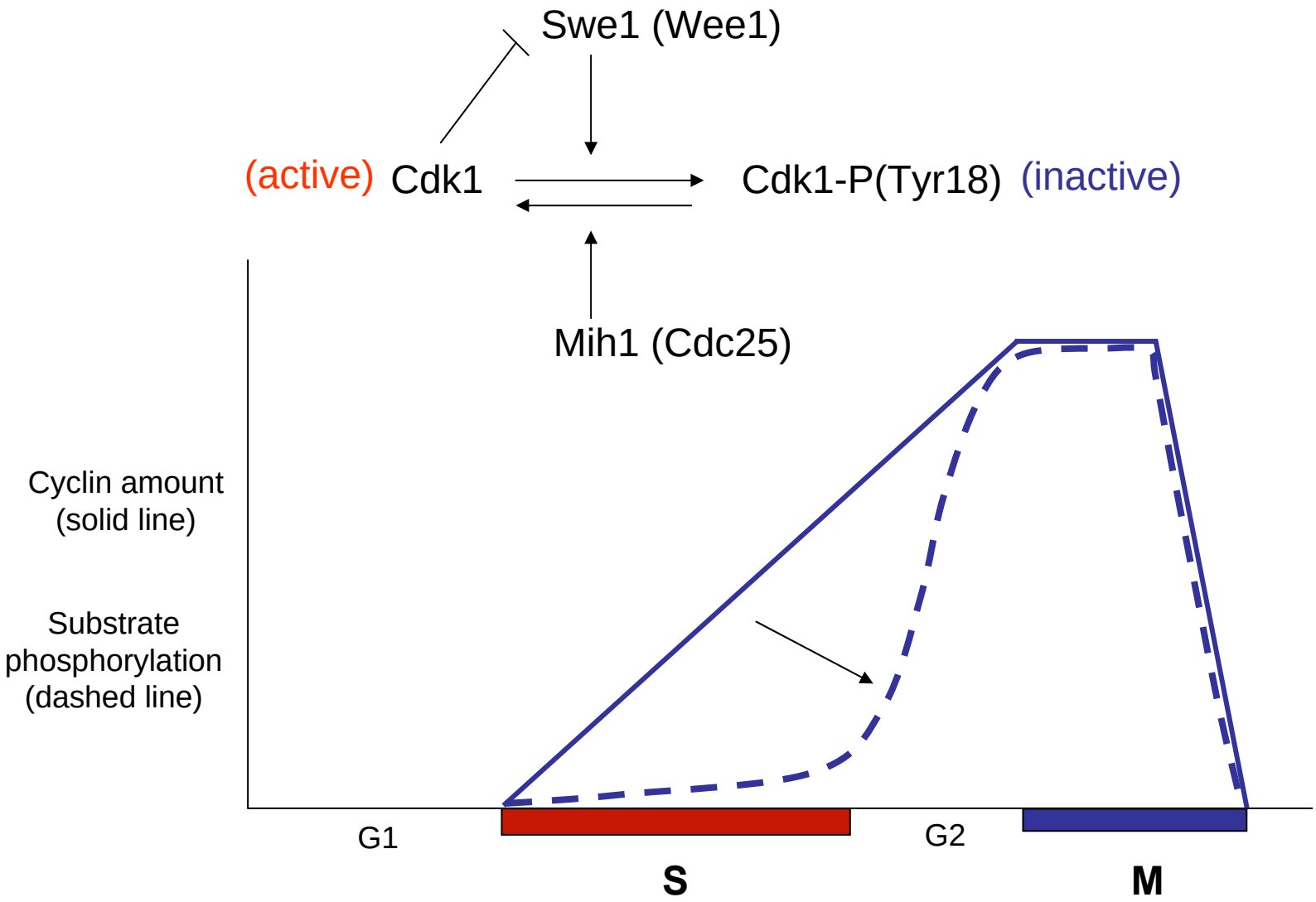
SLOPE: (kcat/Km)/(kcat/Km)η = k3/(k2+k3)

Multiple suboptimal interactions create efficiency and threshold

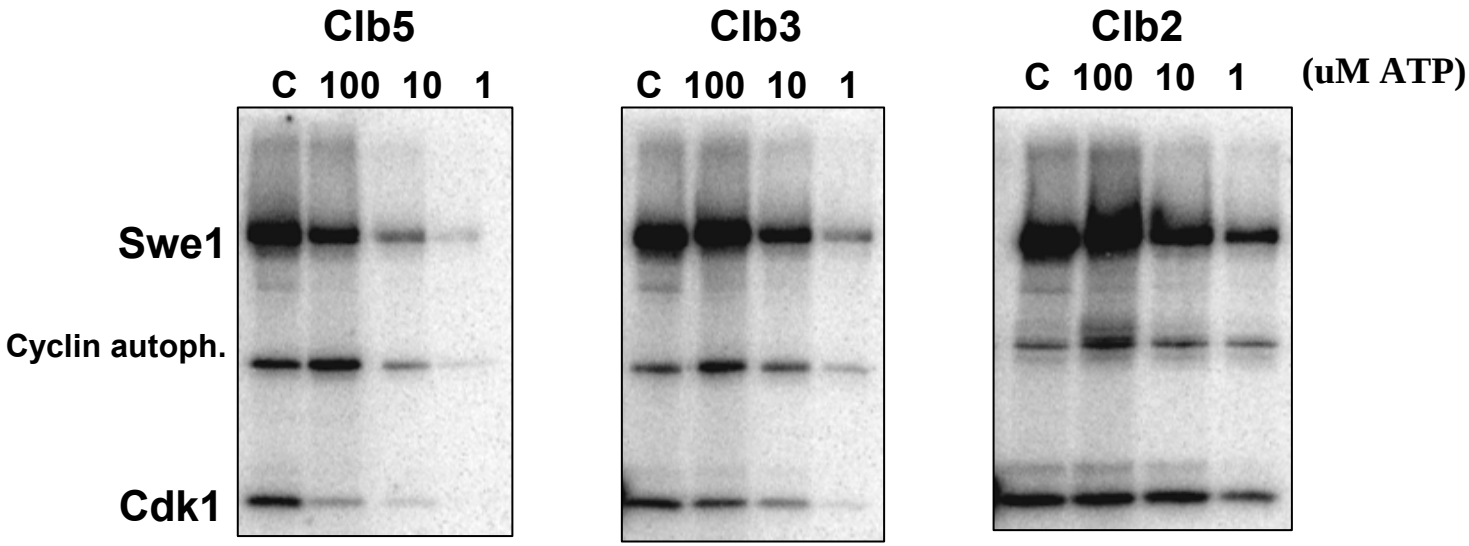


One kinase - three distinct signals




Swe1 (Wee1)
(active) Cdk1
Cdk1-P(Tyr18) (inactive)
Mih1 (Cdc25)
Cyclin amount
(solid line)
Substrate
phosphorylation
(dashed line)
G1
S
G2
M

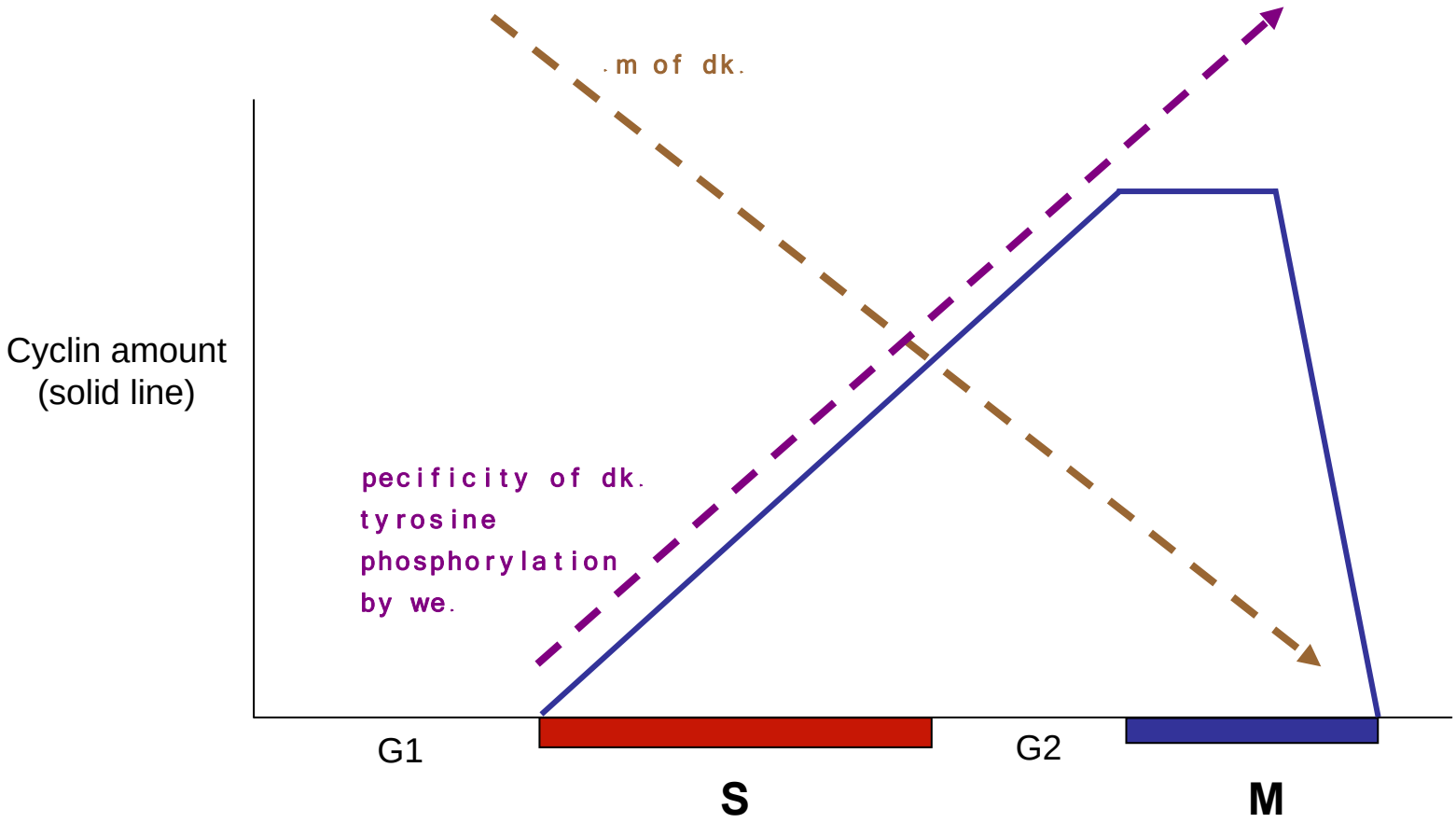
Cyclins modulate the activity of Swe1 toward Cdk1



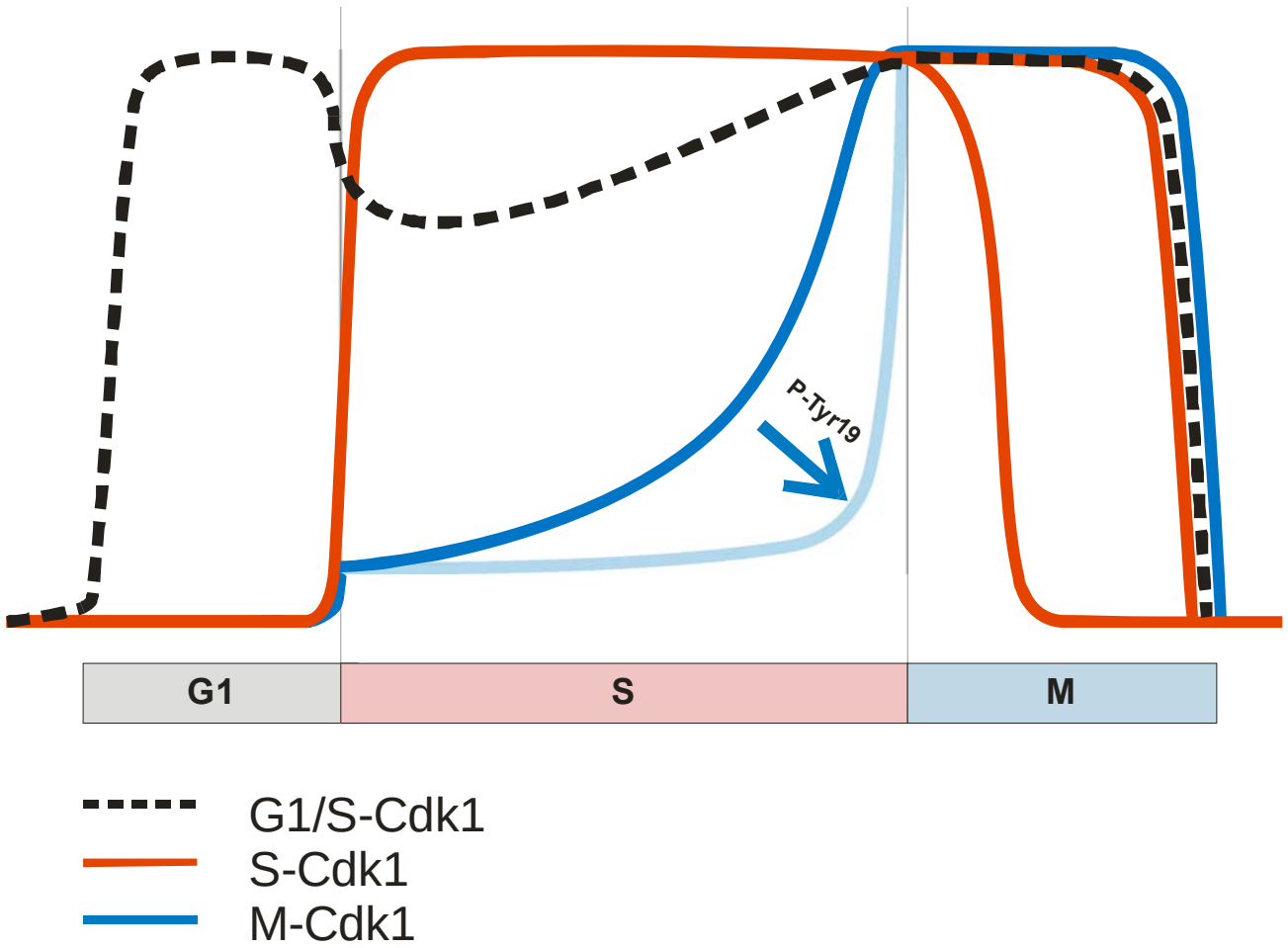
	Cln2	Clb5	Clb3	Clb2
Relative $k_{cat}/K_{M,app}$	0.005<	0.014	0.06	1

G1 → S → M

Periodic cyclin signals introduce gradual changes in Cdk



G2/M transition
Positive feedback via Swe1 downregulation?
Substrate competition: Km1(1+S/Km2)



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