Multistability and hormesis in the dual phosphorylation-dephosphorylation cycle

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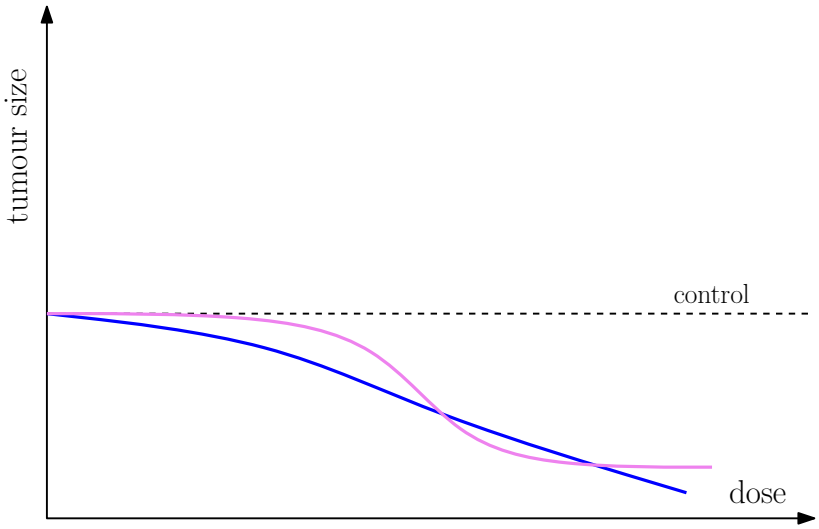
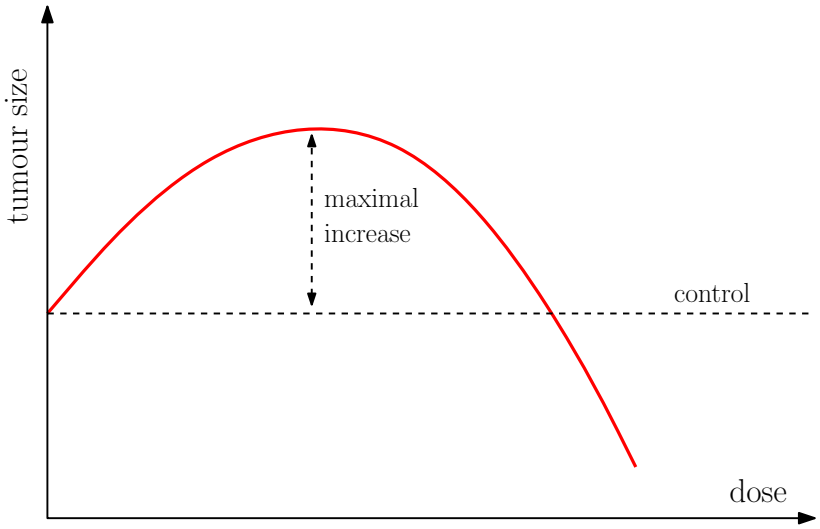
Dose responses for therapeutic compounds

Classical PD models



- threshold (Hill function)
- linear no-threshold
- downward sloping

Hormesis



- inverted U shape, “low-dose response”, “sub-inhibitory concentration”

known from toxicology, growing implications in oncology

Hormesis in oncology

- dexamethasome & growth of neuroepithelian tumour cells[1]
- PACAP-37 & neuroblastoma cell proliferation[2]
- cadmium, mercury ions & MAPK signalling pathway[3]
- RAF inhibitors & RAF kinases in MAPK signalling pathway[4]

[1] Kawamura et al., *Neurol. Med. Chir (Tokyo)* (1998)

[2] Lelievre et al., *Cell Signal.* (1998)

[3] Boldt, Weidle, and Kolch, *Carcinogenesis* (2002)

[4] Hall-Jackson et al., *Chem. Biol.* (1999); Hatzivassiliou et al., *Nature* (2010); Heidorn et al., *Cell* (2010); Poulikakos et al., *Nature* (2010)

Proposed mechanisms for hormesis: overview

- interaction of different receptors which up- and down-regulate a pathway via the same agonist[5]
- superimposition of different dose response curves[6]
- heterogeneous response of different tissues[7]
- overcompensation from disruption[8]
- models with a *"built-in"* feature

[5] Szabadi, *J. Theor. Biol* (1977)

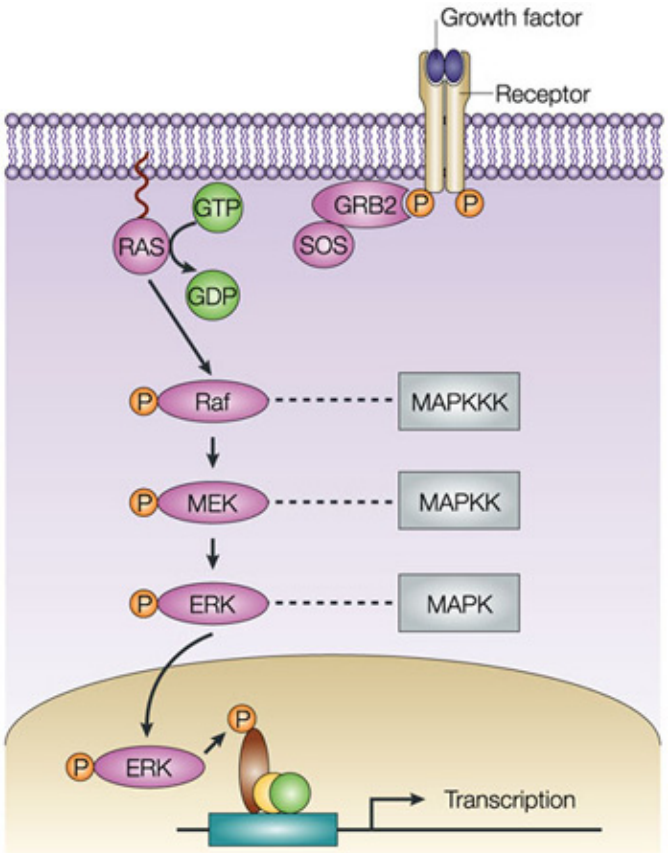
[6] Conolly and Lutz, *Toxicol. Sci.* (2004)

[7] Bae et al., *Toxicol. Sci.* (2008)

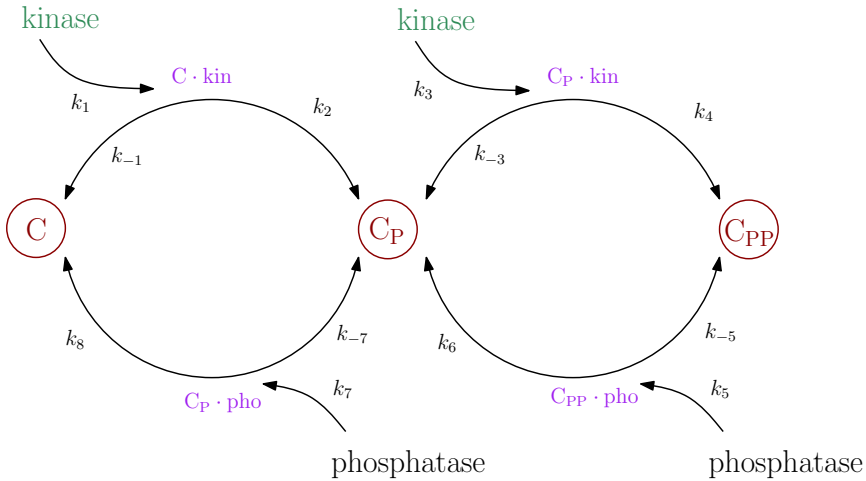
[8] Garzon and Flores, *InTech* (2013)

MAPK signalling pathway

MAPK



Dual phosphorylation-dephosphorylation cycle



activation of MEK, ERK by a distributive mechanism

- two sequential phosphorylation steps
- two sequential dephosphorylation steps

sharing the same intermediate mono-phosphorylated form[9]

[9] Huang and Ferrell, *Proc. Natl. Acad. Sci. USA* (1996)

Image: *Nat. Rev. Mol. Cell Biol.* (2004)

Dual phosphorylation-dephosphorylation cycle

Reaction scheme

$$\mathrm{C} + \mathrm{kin} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{C}\cdot\mathrm{kin} \xrightarrow{k_2} \mathrm{C_P} + \mathrm{kin}$$

$$\mathrm{C_P} + \mathrm{kin} \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} \mathrm{C_P}\cdot\mathrm{kin} \xrightarrow{k_4} \mathrm{C_{PP}} + \mathrm{kin}$$

$$\mathrm{C_{PP}} + \mathrm{pho} \underset{k_{-5}}{\overset{k_5}{\rightleftharpoons}} \mathrm{C_{PP}}\cdot\mathrm{pho} \xrightarrow{k_6} \mathrm{C_P} + \mathrm{pho}$$

$$\mathrm{C_P} + \mathrm{pho} \underset{k_{-7}}{\overset{k_7}{\rightleftharpoons}} \mathrm{C_P}\cdot\mathrm{pho} \xrightarrow{k_8} \mathrm{C} + \mathrm{pho}$$

Properties

- cycle can exhibit *multistability*[10]
- necessary condition[11]: ratio of the catalytic constants $k_2/k_8 < k_4/k_6$
- rigorous proof, conditions on total concentrations of substrate, kinase and phosphatase derived from a high-degree polynomial[12]

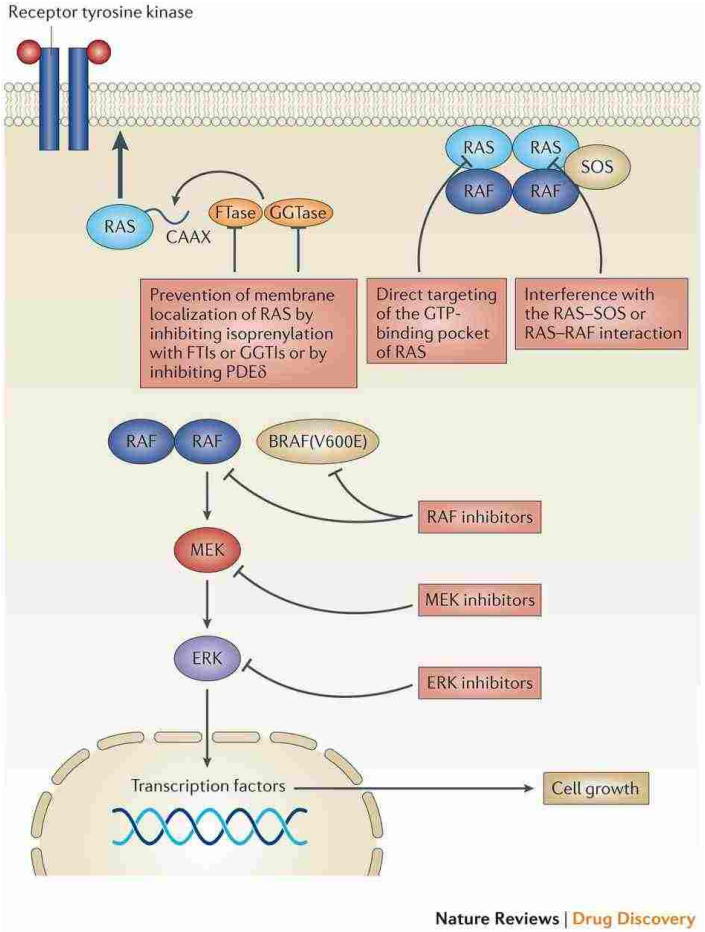
[10] Markevich, Hoek, and Kholodenko, *J. Cell. Biol.* (2004)

[11] Ortega et al, *FEBS J* (2006)

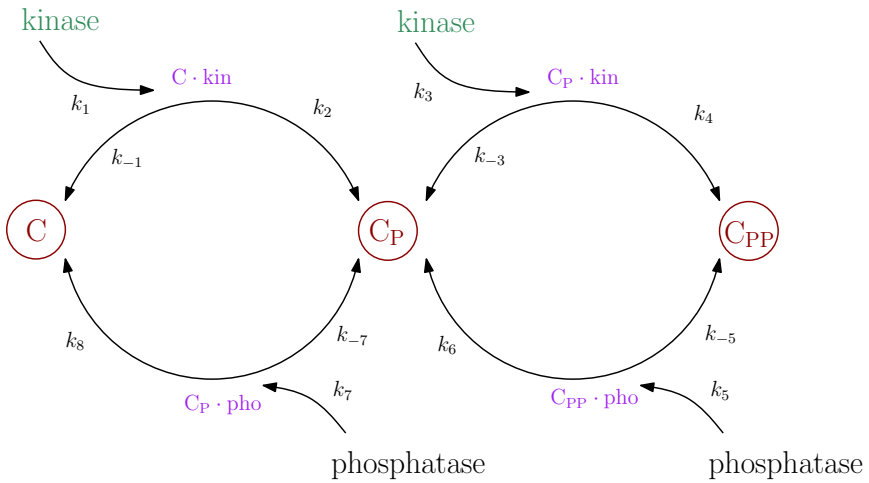
[12] Conradi and Mincheva, *JRS Interface* (2014)

MAPK signalling pathway

MAPK pathway inhibitors



Dual phosphorylation-dephosphorylation cycle



cycle *can exhibit* hormesis of double phosphorylated substrate C_{PP} if targeted with a kinase inhibitor[13]

[13] Rashkov et al, *PLoS Comput. Biol.* (2016)

Image: *Nat Rev Drug Disc* (2014)

Reaction scheme: Hyperbolic inhibition [14]

$$
\begin{array}{ccccccccc}
 & & \mathrm{inh} & & \mathrm{inh} & & & & \\
 & & + & & + & & & & \\
\mathrm{C} & + & \mathrm{kin} & \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} & \mathrm{C}\cdot\mathrm{kin} & \xrightarrow{k_2} & \mathrm{C_P} & + & \mathrm{kin} \\
 & & d_r \uparrow\downarrow d_f & & e_{-1} \uparrow\downarrow e_1 & & & & \\
\mathrm{C} & + & \mathrm{kin}\cdot\mathrm{inh} & \underset{e_2}{\overset{e_{-2}}{\rightleftharpoons}} & \mathrm{C}\cdot\mathrm{kin}\cdot\mathrm{inh} & & & &
\end{array}
$$

$$
\begin{array}{ccccccccc}
 & & \mathrm{inh} & & \mathrm{inh} & & & & \\
 & & + & & + & & & & \\
\mathrm{C_P} & + & \mathrm{kin} & \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} & \mathrm{C_P}\cdot\mathrm{kin} & \xrightarrow{k_4} & \mathrm{C_{PP}} & + & \mathrm{kin} \\
 & & d_r \uparrow\downarrow d_f & & e_{-3} \uparrow\downarrow e_3 & & & & \\
\mathrm{C_P} & + & \mathrm{kin}\cdot\mathrm{inh} & \underset{e_4}{\overset{e_{-4}}{\rightleftharpoons}} & \mathrm{C_P}\cdot\mathrm{kin}\cdot\mathrm{inh} & & & &
\end{array}
$$

[14] Cornish-Bowden, *Fundamentals of Enzyme Kinetics* (1995); Segel, *Enzyme Kinetics* (1993)

Assumptions

- total mass conservation for the substrate, kinase, phosphatase, inhibitor
- mass action kinetics
- 13 ODEs for temporal evolution of concentrations
- 4 linear constrains for the mass conservation
- 9 degrees of freedom: high-dimensional system of nonlinear equations

Solving for the steady-state value C_{PP} in a monostable cycle

- behaviour of steady-state value $C_{PP} := C_{PP}(I_{tot})$
- goal: steady-state value C_{PP} monotone-increasing in I_{tot}
- analytical result: assume $e_{-2}, e_{-4} = 0$; there exist rate constants k_i, e_i, such that when

  (a_1) $e_2 >> e_1, e_4 >> e_3$,
  (a_2) dissociation rate $d_r/d_f >> 1$

  C_{PP} is monotone increasing in I_{tot} (*hormesis*[15])
- if dissociation rates of substrate-kinase-inhibitor complexes $e_2 = e_4 = 0$, steady-state value of C_{PP} is always monotone decreasing in I_{tot}

[15] Rashkov et al, *PLoS Comput. Biol.* (2016)

Solving for the steady-state value C_{PP} (cont'd)

- numerical simulation: there exist rate constants k_i, e_i such that when
  ($a_1^\star$) $e_2, e_4 > 0$,
  (a_2) dissociation rate $d_r/d_f >> 1$,
  C_{PP} is monotone increasing in I_{tot} (*hormesis*[16])
- numerical continuation of the solution from $I_{tot} = 0$
- computations done in MatCont[17]

[16] Rashkov et al, *PLoS Comput. Biol.* (2016)

[17] Dhooge, Govaerts, and Kuznetsov, *ACM Trans Math Software* (2003)

Hormesis in the monostable cycle

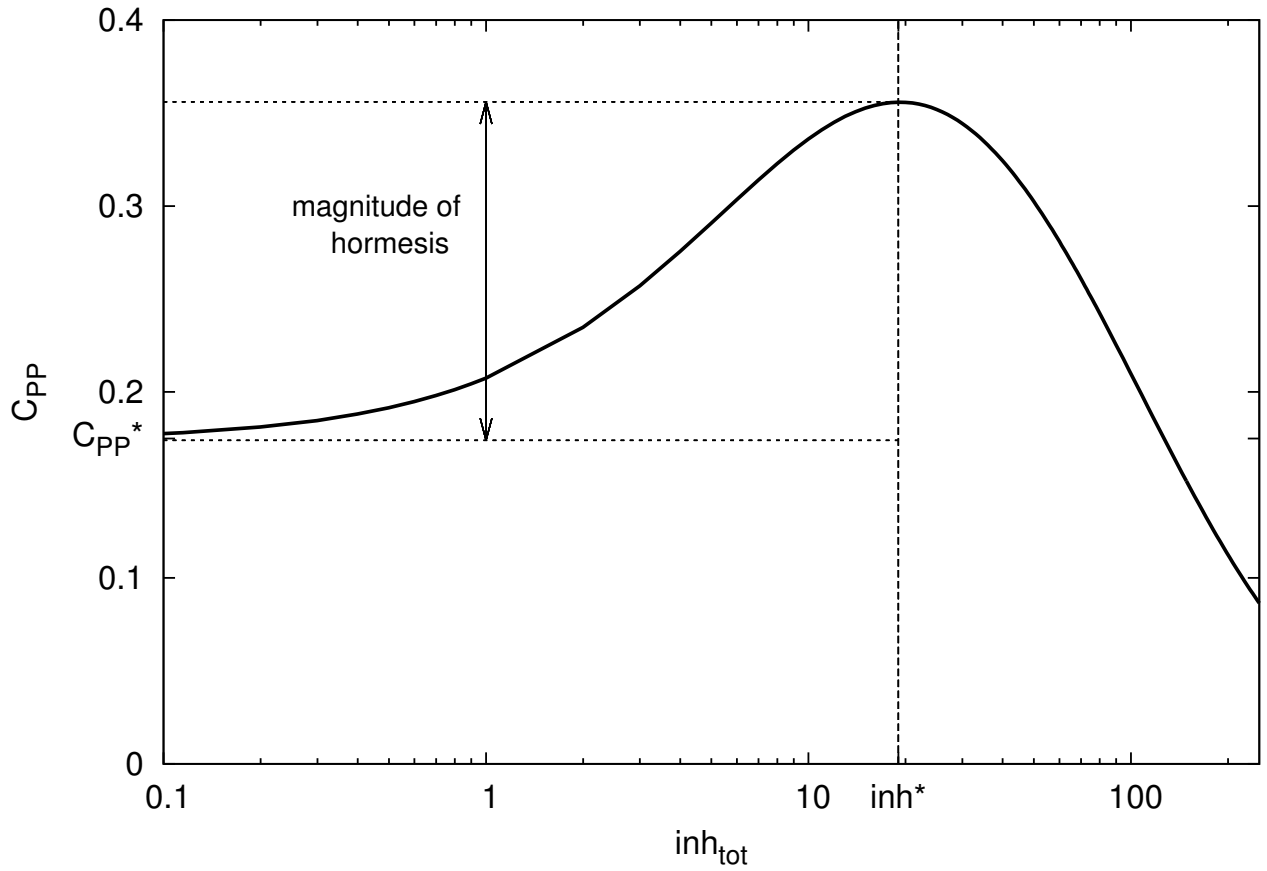


Figure: Inverted U-shape dose-response curve.

Bistable cycle

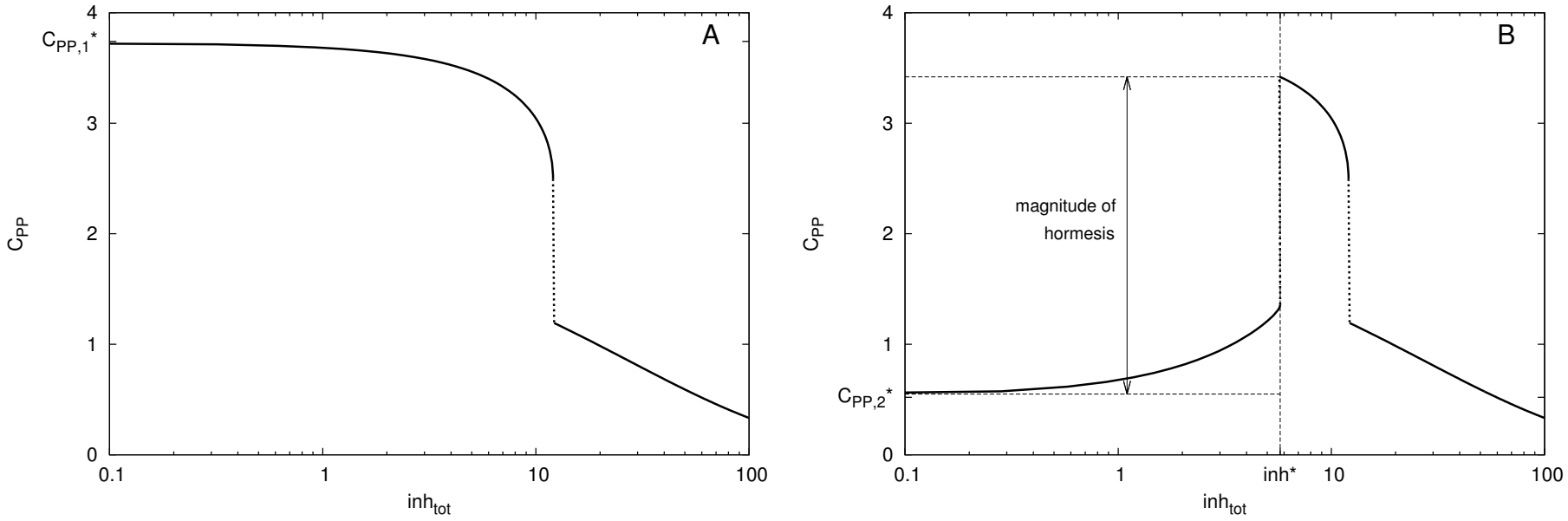


Figure: The dose response curve can be monotone and downward sloping (*panel* A) or has an inverted U-shape characteristic of hormesis (*panel* B).

Magnitude of hormesis

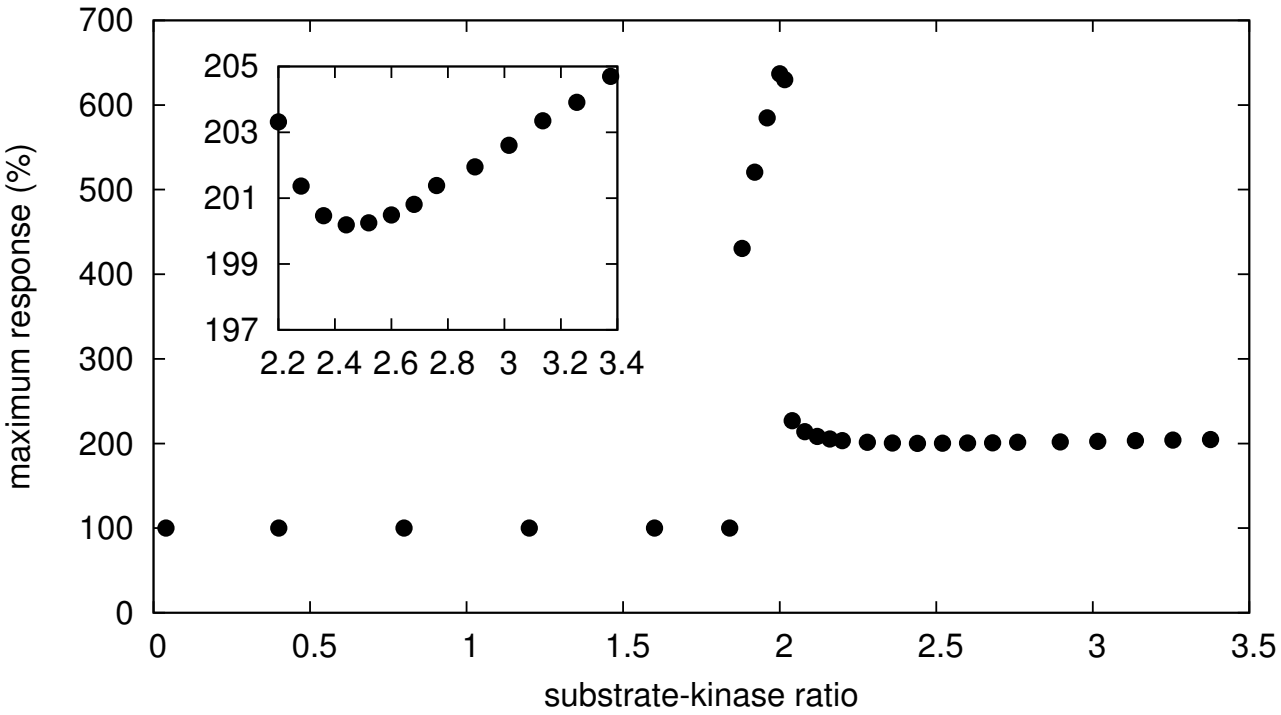
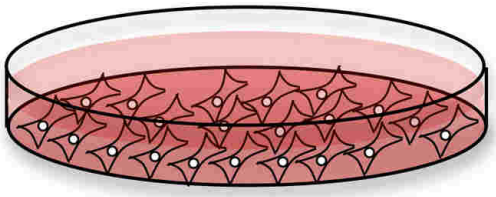
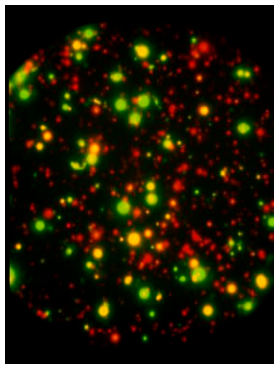


Figure: Maximum response ($\max_{I_{tot}} C_{PP}/C_{PP}(0)$) for different substrate-kinase ratios.

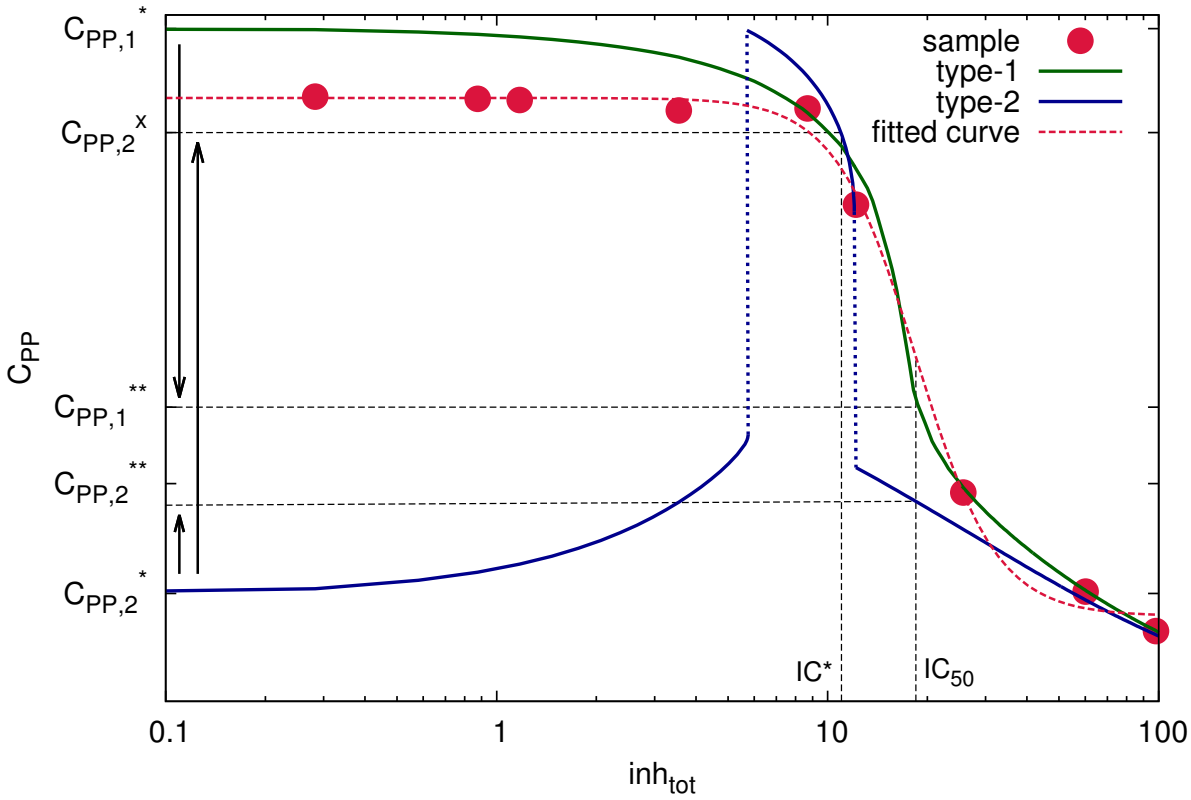
Detection of hormesis at population level

Typical cell assays





Hormesis masked at population level



Measurement from a typical cell assay with two different cell types

Image: ©2010 *Nature Education*; advansta.com; www.ucytech.com

Significance and applications for drug discovery

- MAPK mediates cell proliferation[18]
- "few examples of studies on hormetic responses and cell signalling pathways in tumour cells"[19]
- hormetic effect depends on the catalytic and reaction rate constants k_i, e_i
- rate constants difficult to measure with standard assays, or even non-identifiable
- difficult to distinguish between mono- and double-phosphorylated forms of substrate
- biochemical assays sometimes employ only kinase and inhibitor, but not phosphatase

[18] Roberts and Der, *Oncogene* (2007)

[19] Calabrese, *Crit. Rev. Toxicol* (2013)

Significance and applications for drug discovery

- often in modelling literature the two sequential phosphorylation and dephosphorylation steps are combined

$$\mathrm{C} + \mathrm{kin} \rightleftharpoons \mathrm{C_{PP}} + \mathrm{kin}$$

$$\mathrm{C_{PP}} + \mathrm{pho} \rightleftharpoons \mathrm{C} + \mathrm{pho}$$

- often assumed in biochemical practice that $e_2, e_4 = 0$

$$\mathrm{C} \cdot \mathrm{kin} + \mathrm{inh} \underset{e_{-1}}{\overset{e_1}{\rightleftharpoons}} \mathrm{C} \cdot \mathrm{kin} \cdot \mathrm{inh} \xrightarrow{e_2} \cancel{\mathrm{C} + \mathrm{kin} \cdot \mathrm{inh}}$$

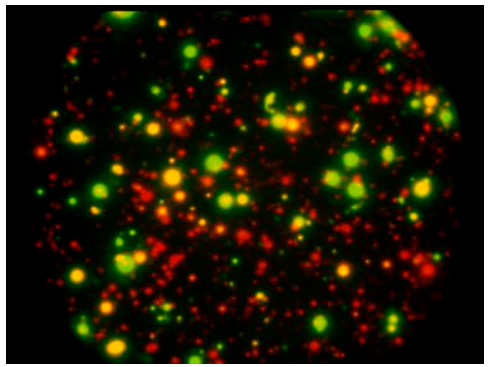
$$\mathrm{C_P} \cdot \mathrm{kin} + \mathrm{inh} \underset{e_{-3}}{\overset{e_3}{\rightleftharpoons}} \mathrm{C_P} \cdot \mathrm{kin} \cdot \mathrm{inh} \xrightarrow{e_4} \cancel{\mathrm{C_P} + \mathrm{kin} \cdot \mathrm{inh}}$$

- simplified computations of rates, but. . . "inhibitor binding need not be dead-ended"[20]

[20] Cornish-Bowden, *Fundamentals of Enzyme Kinetics* (1995)

Significance and applications for drug discovery

- non-genetic heterogeneity[21]
- signalling pathways may exhibit multistability
- not detectable using gene sequencing
- not manifest at cell population level when using a western blot or a fluorescent assay



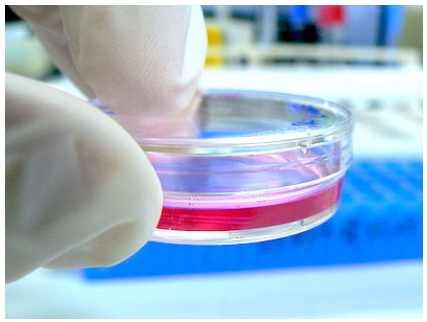
- acceleration of tumour progression, metastasis and drug resistance

[21] Huang, *Cancer Metast. Rev.* (2013); Pisco and Huang, *Brit. J Cancer.* (2013)

Image: advansta.com; www.ucytech.com

Summary

- simple systems with complex behaviour
- non-trivial, paradoxical behaviour from first principles: multistability and hormesis in MAPK
- implications for measurements at population level
- implications for drug discovery



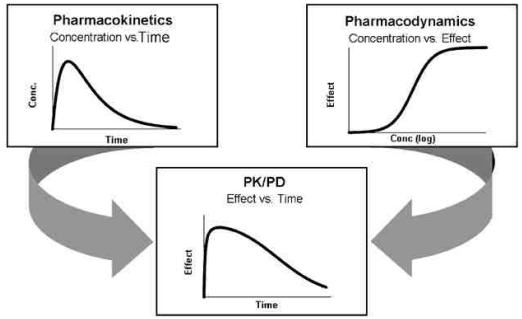


Image: ©Umberto Salvagnin via Wikimedia Commons;
JVPT (2004); www.sethinktank.com

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Merci beaucoup de votre attention!