Supplementary Materials

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Supplementary Materials for

Merging Systems Biology with Pharmacodynamics

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Merging systems biology with pharmacodynamics

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MODEL DESCRIPTION

An operational ePD model for an epidermal growth factor receptor (EGFR) inhibitor was developed based on published mathematical models of the EGFR signaling pathway and biochemical knowledge of the pathway reported in literature. We used the Brightman and Fell model[1] as a framework model for the EGFR stimulation of MAPK/ERK signaling pathway. We simplified the model by consolidating some of the similar reactions and/or by applying Michaelis-Menten kinetics. The modified MAPK/ERK model was extended to incorporate the following key signaling modules: the PLCγ-mediated activation of PKC and its inhibition of RKIP, which limits the catalytic activity of the Raf-1 protein; the downstream ERK-mediated expression of target genes, specifically *CCND1* gene (cyclin D); $p27^{kip}$-mediated inhibition of Cyclin D-CDK4/6 activity- (called active Cyclin D); and mir-221-mediated reduction of $p27^{kip}$ levels. To model drug activity, we assumed that the oncogene was the mutated activated EGFR and that the EGFR inhibitor was a specific tyrosine kinase inhibitor of EGFR that bound directly to EGFR that resulted in a constant fractional inhibition of 80%. To assess tumor size changes (measured in mg) to EGFR inhibition, the amplitude and duration of active Cyclin D was used as an indicator of the rate of cell proliferation measured as the normalized mass doubling time to the growth fraction of the tumor. The key components of the overall ePD model are depicted in Figure 1 in the main text.

To construct the model, we assumed a well-mixed cellular compartment with a cell volume of approximately 1.0×10^{-12} L. Therefore, the resulting model is a system of 34 non-linear ordinary differential equations (ODEs) with 69 kinetic parameters arising from chemical reactions represented mostly by the law of mass action and Michaelis-Menten kinetics. The complexity of the EGF-driven *CCND1* gene expression was approximated by using the Hill function. Many of the initial concentrations and rate constants used in the model were obtained from the previous models including the Brightman and Fell model[1-7]. For the remaining unknown parameters for our proposed molecular reactions, we estimated them by comparing to similar molecular events in EGFR or other signaling pathway models and by choosing arbitrarily parameter values that gave qualitatively similar dynamic responses to those observed from other models[8]. The complete set of the model equations was integrated using the *ode15s* routine of MATLAB R2011b (7.13.0.564) (The MathWorks, Inc.). Complete set of model equations and parameters are shown in Table S1 and2.

The cell signaling model provided with values of active Cyclin D concentration that we used as marker for entry into the cell cycle as suggested by Stacey[9]. We hypothesize that the total area under the active Cyclin D is related to the mass doubling time given the growth fraction by a constant factor, c as 4.8×10^{-6}, which we empirically determined through hypothesizing that the "normal" variation has a rate of 0.1. We then estimated tumor size (mg) from mass doubling time given the growth fraction of the tumor according to Table 3-2 found in Ref #10[10] using the Hill equation with V_{max} of 6.98×10^{3}, K = 0.4884, and n of 1.1802. The resulting tumor sizes for the various conditions are shown in Figure 2 of the main text. This procedure is described as equation 38-39 in Table S3. Various simulation conditions for multiple genomic and epigenetic alterations are specified in Table S4.

Figure S1

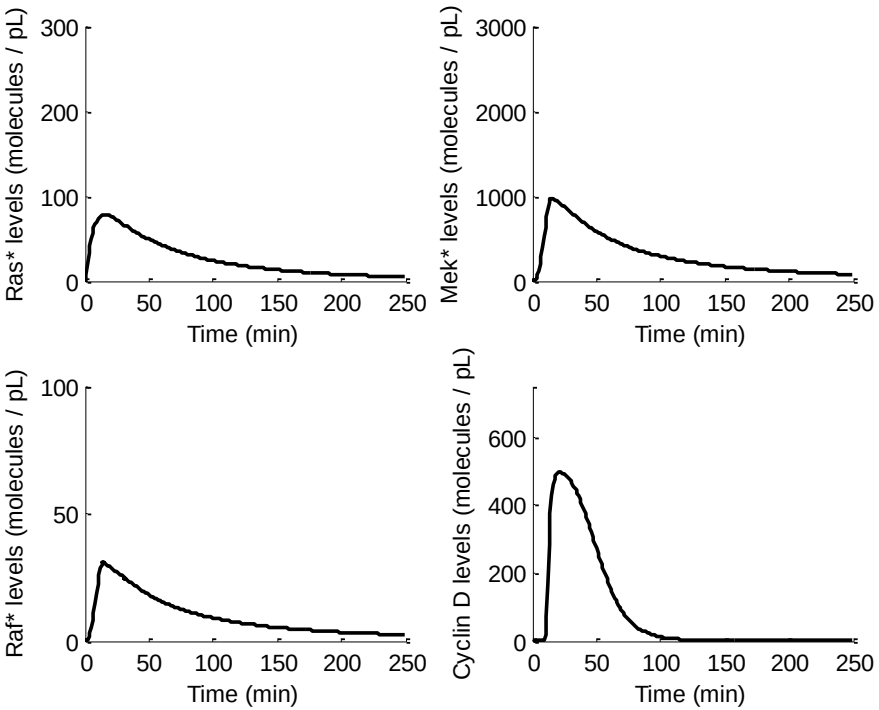

Figure S1: Temporal Profiles of Active Signaling Components of the EGFR pathway in the Standard Patient (SP)
Levels of activated (*) Ras, Raf and Mek and Cyclin D levels in the standard patient receiving EGFR antagonist therapy

Figure S2

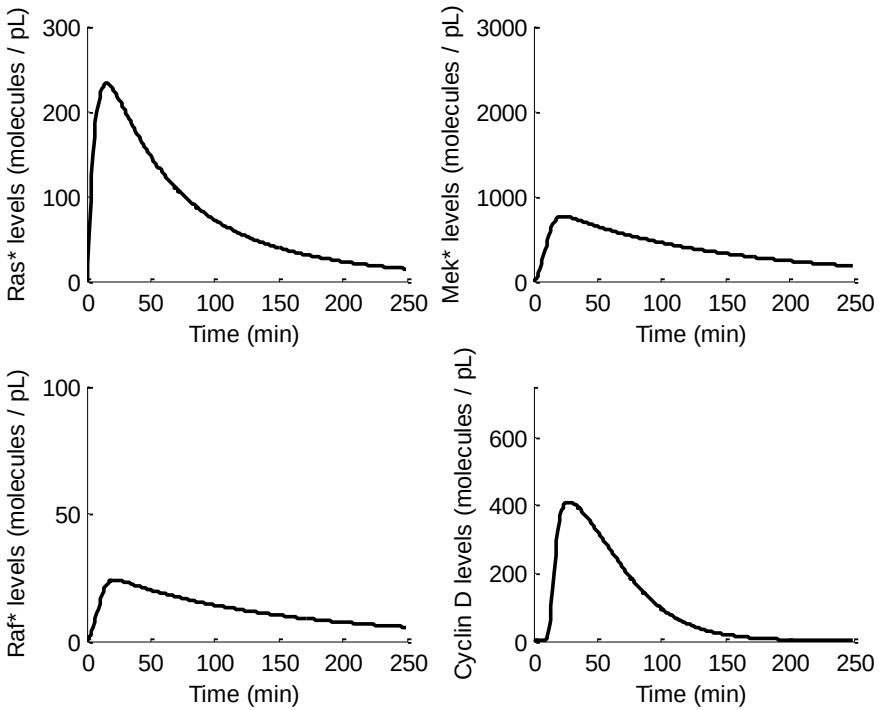

Figure S2: Temporal Profiles of Active Signaling Components of the EGFR pathway in Patient B
Levels of activated (*) Ras, Raf and Mek and Cyclin D levels in Patient B who has hypermethylated *RASAL1* gene resulting in lower levels of RasGAP and a SNP in *RKIP/PEBP1* gene that renders it unresponsive to PKC regulation and is receiving EGFR antagonist therapy

Figure S3

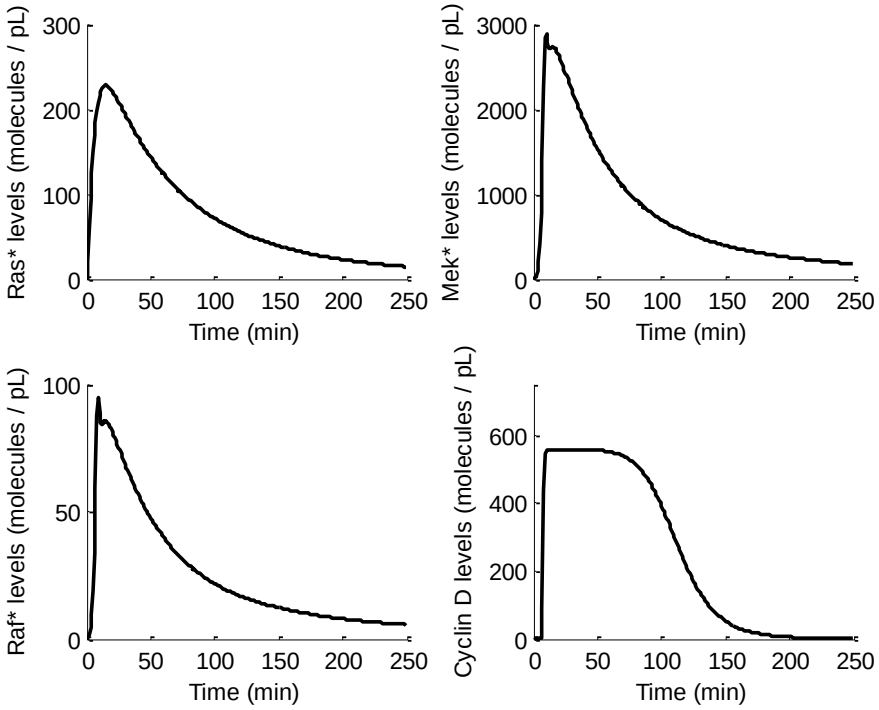

Figure S3 Temporal Profiles of Active Signaling Components of the EGFR pathway in Patient A

Levels of activated (*) Ras, Raf and Mek and Cyclin D levels in Patient A who has hypermethylated *RASAL1* gene that decreases the levels of RasGAP and is receiving EGFR antagonist therapy

Figure S4

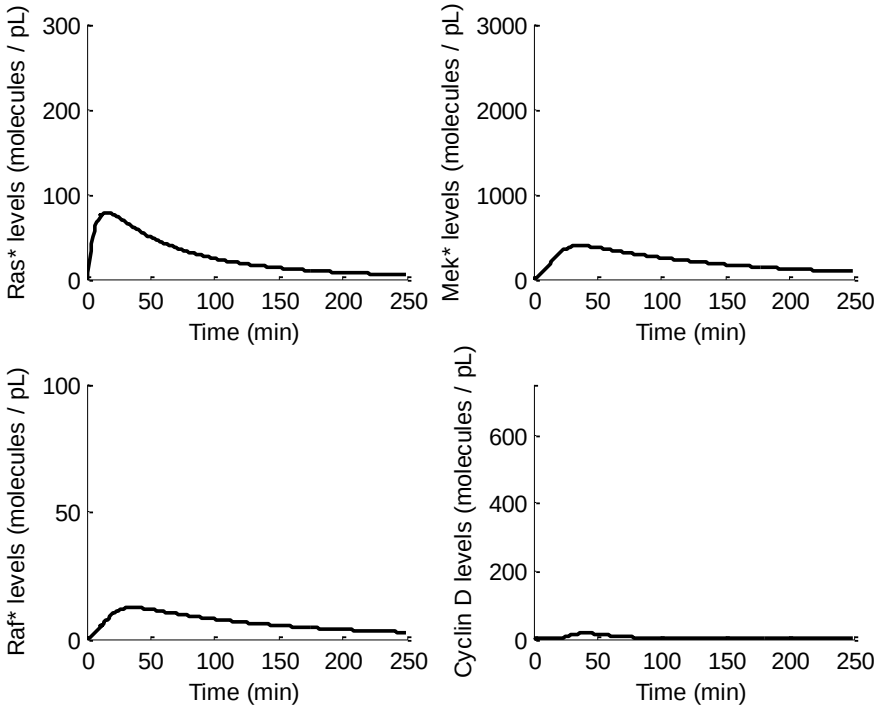

Figure S4 Temporal Profiles of Active Signaling Components of the EGFR pathway in Patient C.
Levels of activated (*) Ras, Raf and Mek and Cyclin D levels in Patient C who has a SNP in *RKIP/PEBP1* gene that renders it unresponsive to PKC regulation and decreased levels of miR-221resulting in increased levels of the cell cycle inhibitor p27kip and is receiving EGFR antagonist therapy.

Table S1. **CHARACTERISTICS OF DIFFERENT TYPES OF COMPUTATIONAL MODELS.**

• **Toy Models**: Arbitrary parameter values are used for both reactant concentrations and reaction rates. Generally toy models are used to make a theoretical point.
• **Plausible Models**: Parameter values that are experimentally measured or estimated from experiments are used to construct these models. Models are generally not specific for any cell type and use parameter values obtained from different cell types and species. These are the most common type of models in Systems Biology.
• **Operational Models**: These models use a combination of experimentally measured or estimated parameters as well as arbitrary parameters to develop a system wherein we can explicitly connect genomic/epigenomic and posttranslational changes to model parameters. For example in the model described here, we have arbitrarily assumed that a two fold increase in promoter region DNA methylation results in a 2 fold decrease in protein levels. This assumption (toy parameter) will have to be experimentally verified in the specific system of interest.
• **Identifiable Models:** These are models built to explain experimental observations. They are system-specific and fully constrained by or fitted to experimental data. These models are the type most commonly used in current PK/PD studies. Often the molecular basis for the experimentally identified parameters is not understood in current PD models.

Table S2. EQUATIONS FOR THE ODE MODEL

Reaction #	Biochemical Reaction	Rate Equations
1	L + R ↔ RL	v1 = ka(1)*R*L - kb(1)*RL
2	RL + RL ↔ RL2	v2 = ka(2)*RL^2 - kb(2)*RL2
3	RL2 ↔ RP	v3 = ka(3)*RL2 - kb(3)*RP
4	RP → RL2	v4 = Vm(4)*RP/(Km(4)+RP)
5	Shc (+ RP) → ShcP (+ RP)	v5 = kc(5)*RP*Shc/(Km(5)+Shc)
6	ShcP → Shc	v6 = Vm(6)*ShcP/(Km(6)+ShcP)
7	ShcP + GrbSos ↔ ShcGrbSos	v7 = ka(7)*ShcP*GrbSos - kb(7)*ShcGrbSos
8	ShcGrbSos (+ ErkP) → GrbSosP + ShcP (+ ErkP)	v8 = kc(8)*ErkP*ShcGrbSos/(Km(8)+ShcGrbSos)
9	GrbSosP → GrbSos	v9 = Vm(9)*GrbSosP/(Km(9)+GrbSosP)
10	ShcGrbSos + RasGDP ↔ ShcGrbSosRas	v10 = ka(10)*RasGDP*ShcGrbSos - kb(10)*ShcGrbSosRas
11	ShcGrbSosRas → RasGTP + ShcGrbSos	v11 = kc(11)*ShcGrbSosRas
12	RasGTP → RasGDP	v12 = kc(12)*GAP*RasGTP/(Km(12)+RasGTP)
13	Raf (+ RasGTP) → RafP (+ RasGTP)	v13 = kc(13)*RasGTP*Raf/(Km(13)+Raf)
14	RafP → Raf	v14 = Vm(14)*RafP/(Km(14)+RafP)
15	Mek (+RafP) → MekP (+RafP)	v15 = kc(15)*RafP*Mek/(Km(15)+Mek)
16	MekP → Mek	v16 = Vm(16)*MekP/(Km(16)+MekP)
17	Erk (+ MekP) → ErkP (+ MekP)	v17 = kc(17)*MekP*Erk/(Km(17)+Erk)
18	ErkP → Erk	v18 = Vm(18)*ErkP/(Km(18)+ErkP)
19	PLCg (+ RP) ↔ PLCgP (+ RP)	v19 = kc(19)*RP*PLCg/(Km(19)+PLCg)

20	PLCgP → PLCg	v20 = Vm(20)*PLCgP/(Km(20) + PLCgP)
21	PKC (+ PLCgP) → PKCP (+ PLCgP)	v21 = kc(21)*PLCgP*PKC/(Km(21)+PKC)
22	PKCP → PKC	v22 = Vm(22)*PKCP/(Km(22)+PKCP)
23	RafP + RKIP ↔ RafPRKIP	v23 = ka(23)*RafP*RKIP - kb(23)*RafPRKIP
24	RafPRKIP (+ PKCP) → RafP + RKIPp (+ PKCP)	v24 = kc(24)*PKCP*RafPRKIP/(Km(24)+RafPRKIP)
25	RKIPp → RKIP	v25 = Vm(25)*RKIPp/(Km(25)+RKIPp)
26	RSK (+ ErkP) → RSKP (+ ErkP)	v26 = kc(26)*ErkP*RSK/(Km(26)+RSK)
27	RSKP → RSK	v27 = kc(27)*RSKP
28	Jun (+ RSKP) → JunP (+ RSKP)	v28 = kc(28)*RSKP*Jun/(Km(28)+Jun)
29	JunP (+ PP1 + PP2A) → Jun (+ PP1 + PP2A)	v29 = kc(29)*PP1*JunP/(Km(29)+JunP) + kc(30)*PP2A*JunP/(Km(30)+JunP)
31	0 → CycD	v31 = Vm(31)*(JunP/Km(31))^kc(31) / (1 + (JunP/Km(31))^kc(31))
32	CycD → 0	v32 = kc(32)*CycD
33	CycD + Kip ↔ CycDKip	v33 = ka(33)*CycD*Kip - kb(33)*CycDKip
34	CycDKip → 0	v34 = ka(34)*CycDKip
35	0 ↔ Kip	v35 = Vm(35) - kb(35)*Kip
36	Kip + uRNA → Kip_uRNA → 0	v36 = ka(36)*Kip*uRNA
37	R + INHB ↔ R_INHB	v37 = ka(37)*R*INHB - kb(37)*R_INHB

ODEs		Initial Conditions [molecule]
LHS d[Species]/dt	RHS	
R	-v1 -v37	5.00E+04
RL	+v1 -v2	0.00E+00
RL2	+v2 -v3	0.00E+00
RP	+v3 -v4	0.00E+00
Shc	-v5 +v6	3.00E+04
ShcP	+v5 -v6 -v7 +v8	0.00E+00
GrbSos	-v7 +v9	2.00E+04
GrbSosP	+v8 -v9	0.00E+00
ShcGrbSos	+v7 -v8 -v10 +v11	0.00E+00
RasGDP	-v10 +v12	1.98E+04
ShcGrbSosRas	+v10 -v11	0.00E+00
RasGTP	+v11 -v12	0.00E+00
Raf	-v13 +v14	1.00E+04
RafP	+v13 -v14 -v23 +v24	0.00E+00
Mek	-v15 +v16	3.60E+05
MekP	+v15 -v16	0.00E+00
Erk	-v17 +v18	7.50E+05
ErkP	+v17 -v18	0.00E+00
PLCg	-v19 +v20	1.05E+04
PLCgP	+v19 -v20	0.00E+00
PKC	-v21 +v22	1.20E+05
PKCP	+v21 -v22	0.00E+00
RKIP	-v23 +v25	1.20E+04
RafPRKIP	+v23 -v24	0.00E+00
RKIPp	+v24 -v25	0.00E+00
RSK	-v26 +v27	6.00E+04
RSKP	+v26 -v27	0.00E+00
Jun	-v28 +v29	2.40E+04
JunP	+v28 -v29	0.00E+00
CycD	+v31 -v32 -v33	0.00E+00
Kip	-v33 +v35 -v36	2.00E+05
CycDKip	+v33 -v34	0.00E+00
INHB	-v37	5.00E+05
R_INHB	+v37	0.00E+00
EGF		1.00E+02 [nM]
miRNA		3.01E+03
PP1	Constant	1.08E+06
PP2A		7.22E+04
GAP		1.50E+04

Table S3. ODE MODEL PARAMETERS. First-order (*kb* and *kc*) and second-order (*ka*) rate constants are given in [min^{-1}] and [$molecule^{-1}\cdot min^{-1}$], respectively, except for *ka*(1) given in [$nM^{-1}\cdot min^{-1}$] and dimensionless *kc*(31). *Vm* and *Km* are expressed in [$molecule\cdot min^{-1}$], and [molecule], respectively.

Reaction #	Parameter				
	ka	*kb*	*kc*	*Vm*	*Km*
1	1.00E-03	7.30E-01			
2	1.38E-03	6.00E+00			
3	6.00E+01	6.00E-01			
4				2.30E+06	3.00E+04
5			1.20E+01		6.00E+03
6				3.00E+05	6.00E+03
7	2.00E-03	3.81E+00			
8			1.60E+00		6.00E+05
9				7.50E+01	2.00E+04
10	1.63E-02	1.00E+01			
11			1.50E+01		
12			7.20E+02		1.56E+05
13			2.70E+01		2.50E+04
14				9.70E+04	6.00E+03
15			5.00E+01		9.00E+03
16				9.20E+05	6.00E+05
17			8.30E+00		9.00E+04
18				2.00E+05	6.00E+05
19			1.80E+01		2.01E+03
20				3.61E+04	6.02E+04
21			3.60E+00		6.02E+04
22				3.61E+04	6.02E+04
23	2.99E-02	3.00E-01			
24			1.80E+02		1.81E+05
25				3.61E+04	6.02E+04
26			1.80E+01		6.02E+03
27			1.80E+01		
28			1.80E+02		1.51E+04
29			1.20E+01		1.81E+04
30			3.60E+01		9.43E+03
31			5.00E+00	1.00E+04	1.50E+03
32			3.60E+00		
33	1.20E-02	6.00E-01			
34	6.00E+00				
35		6.00E+00		1.20E+06	
36	2.99E-01				
37	5.98E-03	7.20E+02			

Table S4. RELATIONSHIP BETWEEN FREE CYCLIN D, MASS DOUBLING GIVEN GROWTH FRACTION, AND TUMOR SIZE.

Equation	Parameter			
	c	*b*	*k*	*n*
Mass Doubling/Growth Fraction (M/G) = c * CycD	4.8E-06			
Tumor Size = $b*(M/G)^n/(K^n+(M/G)^n)$		6.98E+03	0.4884	1.1802

Table S5. SIMULATION CONDITIONS FOR MULTIPLE GENOMIC/EPIGENOMIC ALTERATIONS IN THE EGFR NETWORK.

Genomic/Proteomic Variation	Notation	Condition
CNV or activating mutation in EGFR	E+	2-fold increase in the normal EGFR level
Hypomethylated RASAL1	R+	3-fold increase in the normal GAP level
Hypermethylated RASAL1	R-	3-fold decrease in the normal GAP level
SNP in *PEBP*	K+	100-fold decrease in the affinity of RKIP for PKC
miR221 overexpression	M+	3-fold increase in the normal miR221 level resulting in a proportional increased rate of p27kip mRNA degradation and subsequent decrease in p27kip protein level
miR221 underexpression	M-	3-fold decrease in the normal miR221 level resulting in a proportional decreased rate of p27kip mRNA degradation and subsequent increase in p27kip protein level

REFERENCES

1. Brightman, F.A. and D.A. Fell, *Differential feedback regulation of the MAPK cascade underlies the quantitative differences in EGF and NGF signalling in PC12 cells.* FEBS Letters, 2000. **482**: 169-174.
2. Cho, K.-H., et al., *Mathematical Modeling of the Influence of RKIP on the ERK Signaling Pathway*, in *Lecture Notes in Computer Science: Computational Methods in Systems Biology*. 2003, Springer. p. 127-141.
3. Kwon, H., et al., *Determination of binding constant of transcription factor AP-1 and DNA.* European Journal of Biochemistry, 2001. **268**. 565-572.
4. Kholodenko, B.N., et al., *Quantification of short term signaling by the epidermal growth factor receptor.* J Biol Chem, 1999. **274**:30169-30181.
5. Novak, B. and J.J. Tyson, *A model for restriction point control of the mammalian cell cycle.* J Theor Biol, 2004. **230** 563-579.
6. Ajay, S.M. and U.S. Bhalla, *A role for ERKII in synaptic pattern selectivity on the time-scale of minutes.* European Journal of Neuroscience, 2004. **20**: 2671-2680.
7. Zhou, W., et al., *Novel mutant-selective EGFR kinase inhibitors against EGFR T790M.* Nature, 2009. **462**: 1070-1074.
8. Schoeberl, B., et al., *Computational modeling of the dynamics of the MAP kinase cascade activated by surface and internalized EGF receptors.* Nat Biotechnol, 2002. **20**. 370-375.
9. Stacey, D.W. *Cyclin D1 serves as a cell cycle regulatory switch in actively proliferating cells.* Current Opinion in Cell Biology 2003 **15** 158-163 .
10. Pratt, W.B. *The anticancer drugs.* pp 29 (Oxford University Press: 1994). at http://books.google.com/books?id=nPR1L4K5HuEC&pgis=1