Supplementary Text S1: Modeling and computational details

'Kinetic Modeling and Analysis of the Akt/Mechanistic Target of Rapamycin Complex 1 (mTORC1) Signaling Axis Reveals Cooperative, Feedforward Regulation,' by Anisur Rahman and Jason M. Haugh

Kinetic model of the Akt/mTORC1 signaling axis

We describe in mathematical terms the biochemical mechanisms by which stimulation of cells with PDGF elicits activation of mTORC1 and the ensuing phosphorylation of S6K1. Considering the granularity and emphasis of the model we aimed to develop, we chose not to model the PDGF receptor dynamics (ligand binding, receptor dimerization, receptor trafficking, and ligand depletion) in detail. Rather, the level of activated receptors is represented by a dimensionless signal function, $\sigma(t)$ (1, 2).

$$\sigma(t) = P(1 - e^{-k_1 t}) + A(e^{-k_1 t} - e^{-k_2 t})$$

This equation has four adjustable parameters: *P* representing the plateau (steady-state) value of $\sigma(t)$, *A* related to the peak value of $\sigma(t)$, rate constant k_1 describing the decay of $\sigma(t)$, and rate constant $k_2 < k_1$ describing the initial increase of $\sigma(t)$. For each of the four PDGF doses, *P* and *A* are assigned different values, whereas the values of k_1 and k_2 were the same for all doses; thus, there are 10 adjustable parameters that describe the receptor dynamics.

Following previous work (2, 3), we assume that the reversible recruitment of PI3K enzyme by the receptor (fractional activation *e*) is relatively rapid (and therefore near equilibrium) and saturable. No additional parameter is needed here, because the *P* and *A* values in the σ function are freely adjustable; i.e., they incorporate the equilibrium constant of the PI3K-receptor interaction.

$$e = \frac{\sigma}{1 + \sigma}$$

Next is the dynamic equation for phosphoinositide generation. Following previous work (4, 5), we assume that the PI3K reaction is insensitive to the availability of the substrate, phosphatidylinositol (4,5)-bisphosphate, and that the PI 3-phosphatase activities are constitutive and far from saturated. The dimensionless variable *l* represents the 3' PI lipid density scaled by its maximum (i.e., if PI3K were fully recruited), and the rate constant k_{3PI} characterizes the time scale of 3' PI turnover (4, 5).

$$\frac{dl}{dt} = k_{3PI}(e - l); \qquad l(0) = 0$$

The fraction of Akt in the phosphorylated (active) state, *a*, responds to 3' PI. Phosphorylation is modeled as a bimolecular reaction with rate constant k_a, and therefore the activities of the kinases involved are implicit. This is reasonable if one considers binding of 3' PI by the pleckstrin homology domain of Akt as the rate-determining step (6). A pseudo-first-order rate law, with rate constant k_{-a}, describes dephosphorylation of Akt.

$$\frac{da}{dt} = k_a l(1 - a) - k_{-a} a; \qquad a(0) = 0$$

TSC2 and PRAS40 are phosphorylated by Akt, resulting in the neutralization of these regulators. In the model, *g* and *p* are the fractions of TSC2 (GAP) and PRAS40, respectively,

in the phosphorylated state. To account for basal phosphorylation, both Akt-dependent and constitutive phosphorylation terms are included. Akt is assumed to be far from saturation, and Akt dephosphorylation is assumed constitutive. These equations thus introduce the 6 rate constants k_{g0}, k_g, k_{-g}, k_{p0}, k_p, and k_{-p}. The initial conditions are set such that the system is stationary for $a = 0$.

$$\frac{dg}{dt} = \left(k_{g0} + k_g a\right)(1 - g) - k_{-g} g; \qquad g(0) = g_0 = \frac{k_{g0}}{k_{g0} + k_{-g}}$$
$$\frac{dp}{dt} = \left(k_{p0} + k_p a\right)(1 - p) - k_{-p} p; \qquad p(0) = p_0 = \frac{k_{p0}}{k_{p0} + k_{-p}}$$

The model assumes that the small GTPase Rheb is converted to the active, GTP-bound state (fraction r) by a constitutive exchange reaction with pseudo-first-order rate constant k_r. Control is imposed by deactivation of TSC2 GAP activity. The interaction between unphosphorylated GAP (fraction $1 - g$) and Rheb-GTP is assumed to be far from saturation (catalytic efficiency k_{-r}). The initial condition is set such that the system is stationary for $g = g_0$.

$$\frac{dr}{dt} = k_r(1 - r) - k_{-r}(1 - g)r; \qquad r(0) = r_0 = \frac{k_r}{k_r + k_{-r}(1 - g_0)}$$

The dimensionless mTORC1 activity, m, is affected positively by Rheb-GTP and negatively by unphosphorylated PRAS40 (dimensionless r and $1 - p$, respectively). To model this regulation in a compact manner, we consider mTOR to be in pseudo-equilibrium with those two entities, noting that the 'interaction' with Rheb-GTP could represent physical binding of Rheb-GTP or an indirect yet proportional effect of Rheb exerted on the complex, e.g. by affecting substrate binding. Taking M_0, M_r, M_p, and M_{rp} as the fractions of mTORC1 in the 'free', Rheb-associated, PRAS40-bound, and both Rheb- and PRAS40-associated states, and assuming that Rheb-GTP and PRAS40 are in excess, the governing equations are as follows.

$$M_r = K_r r M_0; \qquad M_p = K_p(1 - p)M_0;$$
$$M_{rp} = \beta K_r r M_p = \beta K_p(1 - p)M_r = \beta K_r K_p r(1 - p)M_0$$
$$M_0 + M_r + M_p + M_{rp} = 1$$

The parameters K_r and K_p are the associated equilibrium constants, and β is a parameter characterizing the cooperativity of the two interactions. Thus, the model allows for the nature of these interactions to range from mutually exclusive ($\beta = 0$) to completely independent ($\beta = 1$) to positively cooperative ($\beta > 1$). Finally, assuming different contributions to the mTOR activity, with $m = \alpha M_r + (1 - \alpha)M_{rp}$ (constant parameter $0 \leq \alpha \leq 1$), we obtain the following expression.

$$m = \frac{\alpha K_r r + (1 - \alpha)\beta K_r K_p r(1 - p)}{1 + K_r r + K_p(1 - p) + \beta K_r K_p r(1 - p)}$$

Lastly, the kinetic equation for S6K1 phosphorylation, fraction s, is as follows, introducing the rate constants k_s and k_{-s}. The initial condition is such that the equation is stationary for $m = m_0$, where m_0 is the value of m calculated with $r = r_0$ and $p = p_0$.

$$\frac{ds}{dt} = k_s m(1 - s) - k_{-s} s; \qquad s(0) = \frac{k_s m_0}{k_s m_0 + k_{-s}}$$

Acquisition of model parameter set ensembles

We implemented a Monte Carlo routine based on the Metropolis algorithm (7, 8) to align the model to our data and thus identify sets of model parameter values that fit the data almost optimally. The algorithm was implemented in MATLAB (MathWorks), adapted from code described in detail previously (2). A summary of the method follows.

1. An initial set of parameters k_i is chosen. For the results shown in the paper, the initial values were all 0.1 (we confirmed that initial values of 1, or random initial values, yielded approximately the same results). Using these initial values, the dimensionless model output is computed.
2. The model output is aligned to the means of the experimental data by scaling the model output by alignment factors for each readout *j* (phospho-Akt, phospho-TSC2, phospho-PRAS40 and phospho-S6K1). For each parameter set *i*, the value of each alignment factor *j* is chosen to minimize the sum of squared deviations, SSD_{ij}. This is achieved via a branch-and-bound subroutine. In calculating the *SSD*, we weighted the data for different PDGF doses by the following factors, to offset differences in the magnitudes of the data values: 1 for 0.3 and 1 nM PDGF, 2 for 0.03 nM PDGF, and 5 for 0.01 nM PDGF.
3. The cumulative sum of squared deviations, $cSSD_i$, is calculated as follows.
$$cSSD_i = \sum_j SSD_{ij}$$
4. Each parameter k_i is updated according to the following equation.
$$k_{i+1} = k_i(1 + \Delta\mu \,\mathrm{randn})$$
where randn is a random number drawn form a standard normal distribution. For this study, the value of the parameter $\Delta\mu$ was 0.03. The step is redone if k_{i+1} is chosen to be less than 10^{-4} or greater than 10^4.
5. Steps 2 and 3 are repeated for the new parameter set.
6. If $cSSD_{i+1} < cSSD_i$, the new parameter set is accepted, and *i* is incremented. Otherwise, it might still be accepted, with a probability given by the following formula.
$$P_{i+1} = exp\left[-\frac{(cSSD_{i+1} - cSSD_i)}{T_i}\right]$$
$$T_i = T^0 cSSD_i$$
The value of T_i is called the 'temperature', which determines how forgiving the algorithm is when the fit fails to improve. The parameter T^0 is the 'standard' temperature (corresponding to $cSSD_i = 1$), which was set to a value of 0.01 for this study. I.e., a parameter set with much greater than 1% higher *cSSD* than its predecessor is severely penalized. If the new parameter set is rejected, the procedure is repeated using the previous parameter set as the input.
7. The algorithm is run for a sufficient long time, until at least 50,000 parameter sets are accepted. From these, we selected the 10,000 parameter sets with the lowest $cSSD_i$ to comprise the parameter set ensemble used to generate modeling results.

For each parameter set in the ensemble, the model was recalculated, and the relevant outputs were scaled by their respective alignment factors for comparison to the experimental data. The scaled model output for each time course is reported as the ensemble mean ± standard deviation, allowing visual assessment of the accuracy and precision of the fit. A

summary of the distributions for the 27 parameters of the main model is given in the table below.

Parameter	Definition	Min	Q1	Median	Q3	Max
P (0.01)	Signal plateau, 0.01nM PDGF	0.0001	0.00261	0.00347	0.00423	0.00915
A (0.01)	Signal amplitude, 0.01nM PDGF	0.0658	0.162	0.209	0.317	0.674
P (0.03)	Signal plateau, 0.03nM PDGF	0.00168	0.00653	0.00833	0.0104	0.0188
A (0.03)	Signal amplitude, 0.03nM PDGF	0.695	1.51	1.86	2.86	6.96
P (0.3)	Signal plateau, 0.3nM PDGF	0.108	0.245	0.303	0.347	0.560
A (0.3)	Signal amplitude, 0.3nM PDGF	3.01	7.20	9.07	13.6	32.9
P (1)	Signal plateau, 1nM PDGF	0.116	0.262	0.325	0.375	0.587
A (1)	Signal amplitude, 1nM PDGF	7.38	24.9	31.7	55.9	143
k_1	Rate constant, receptor activity decay	0.0657	0.0876	0.0954	0.106	0.170
k_2	Rate constant, receptor activity onset	0.0910	0.122	0.137	0.155	0.288
k_{3PI}	Rate constant, 3' PI turnover	0.0768	0.193	0.238	0.300	0.489
k_a	Rate constant, Akt phosphorylation	0.00319	0.0146	0.0256	0.0969	0.209
k_{-a}	Rate constant, Akt dephosphorylation	0.110	0.279	0.332	0.415	1.06
k_{g0}	Rate constant, basal TSC2 phosph.	0.0133	0.0225	0.0262	0.0314	0.0595
k_g	Rate constant, TSC2 phosph. by Akt	3.44	16.8	32.3	52.1	143
k_{-g}	Rate constant, TSC2 dephosph.	0.114	0.199	0.231	0.276	0.509
k_{p0}	Rate constant, basal PRAS40 phosph.	0.0111	0.0294	0.0379	0.0498	0.146
k_p	Rate constant, PRAS40 phosph. by Akt	7.57	47.7	107	162	395
k_{-p}	Rate constant, PRAS40 dephosph.	0.0350	0.104	0.137	0.184	0.432
k_r	Rate constant, Rheb-GDP → -GTP	0.0007	0.00422	0.00604	0.00903	0.0401
k_{-r}	Rate constant, Rheb-GTP hydrolysis	0.039	0.189	0.304	0.427	1.08
K_r	Equilibrium constant, Rheb/mTOR	1.51	12.6	16.8	38.4	99.2
K_p	Equilibrium constant, PRAS40/mTOR	108	520	781	2.78e3	5.27e3
k_s	Rate constant, S6K1 phosph.	0.612	3.32	4.79	6.37	14.6
k_{-s}	Rate constant, pS6K1 dephosph.	0.0245	0.0473	0.0582	0.0706	0.143
β	mTORC1 cooperativity parameter	0.0001	0.000197	0.000292	0.000818	0.00779
α	mTORC1 activity parameter	0.235	0.784	0.888	0.951	0.999

Statistics of the parameter set ensemble for the main model of the Akt/mTORC1 signaling axis. All parameters labeled as rate constants have units of min^{-1}; all others are dimensionless. For each parameter, the minimum (Min), first quartile (Q1), median, third quartile (Q3), and maximum (Max) of the 10,000 values in the ensemble are listed.

Variations of the model tested

Along with the main model described in the previous section, two variations were tested; we refer to these 'control' models as Rheb only and PRAS40 only. In these variations, the equation describing the regulation of mTOR (species *m*) is replaced by a differential equation as follows.

$$\frac{dm}{dt} = k_m r(1-m) - k_{-m} m; \qquad m(0) = \frac{k_m r_0}{k_m r_0 + k_{-m}} \qquad \text{(Rheb only)}$$

$$\frac{dm}{dt} = k_m (1-m) - k_{-m}(1-p) m; \qquad m(0) = \frac{k_m}{k_m + k_{-m}(1-p_0)} \qquad \text{(PRAS40 only)}$$

Thus, in the Rheb only model it is assumed that mTOR is simply activated by Rheb; modulation of PRAS40 is neglected. Conversely, in the PRAS40 only model, mTOR is simply activated through neutralization of PRAS40. The table below shows a comparison of how well the main model and these two model variations fit the data. Cumulative SSD values of each readout (Akt, TSC2, PRAS40 and S6K1), reported as mean ± s.d. for the ensemble, are reported; a lower *cSSD* indicates a closer overall fit. The main model consistently outperforms the two variations in terms of the cSSD values for each readout. Finally, a comparison index that penalizes models having more adjustable parameters, the Akaike Information Criterion (AIC) (9), was calculated for each model (lower AIC is better). The main model and PRAS40 only model have approximately the same mean AIC values, despite the main model having 4 more adjustable parameters. That said, the subsequent measurements in Rheb- and TSC2-depleted cells show that the underlying assumption of the PRAS40 only model is false.

Also shown in the table are the results of an alternative fit of the main model, with the parameter constraints α = 1, β = 1 (as discussed in the main text). This corresponds to the scenario where Rheb-GTP and PRAS40 engage mTORC1 independently, with PRAS40 canceling the Rheb-induced activation of mTORC1.

	Main model	**Rheb only**	**PRAS40 only**	**α = 1, β = 1**
$cSSD_{Akt}$	2.93 ± 0.40	4.34 ± 0.39	3.23 ± 0.40	3.93 ± 0.37
$cSSD_{TSC2}$	2.48 ± 0.26	3.74 ± 0.44	2.46 ± 0.25	2.34 ± 0.23
$cSSD_{PRAS40}$	1.17 ± 0.15	1.01 ± 0.15	1.52 ± 0.17	1.32 ± 0.21
$cSSD_{S6K1}$	2.56 ± 0.24	3.84 ± 0.41	2.77 ± 0.27	3.57 ± 0.36
AIC (N=84)	106.2	131.1	105.5	118.9
# of parameters (*k*)	27	25	23	25

Table S2: Model comparisons. Cumulative SSD values for each readout (ensemble mean ± s.d.) and the Akaike Information Criterion (AIC) value is listed for each model. The formula for AIC is $AIC = N\,[1 + \ln(2\pi) + \ln(cSSD/N)] + 2k$, where N is the number of observations and k is the number of parameters.

Predictions based on shRNA-mediated depletion of regulatory proteins

To predict how system dynamics are affected by depletion of regulatory proteins, certain parameters were adjusted across the ensemble. For example, to represent 80% knockdown of Rheb, the parameter K_r was reduced by 80%; i.e., the value of K_r in each parameter set was multiplied by 0.2. Likewise, for TSC2 and PRAS40 knockdown, the parameters adjusted are k_{-r}

and K_p, respectively. Then the estimated pS6K1 time course was computed for each parameter set (with the previously determined alignment factor applied), and the ensemble mean constitutes the model prediction. To allow the model prediction to be compared to the corresponding experiment, both were normalized so that the mean of the control (for the same time points) equals 1.

Steady-state analysis based on fixed Akt activity

In this analysis, the Akt activity fraction, *a*, was fixed at various values from 0 to 1 and was considered as the input. The corresponding steady-state values of *g*, *p*, *r*, and *m* were derived by setting the right-hand sides of the differential equations equal to zero and simplifying the resulting algebraic equations. These equations were solved for each parameter set to generate ensemble predictions.

Mathematical analysis of mTORC1 regulation by PRAS40 and Rheb-GTP

The model considers two distinct ways for PRAS40 to antagonize Rheb-induced mTORC1 activity: through binding competition ($\beta < 1$) or through modulation of catalytic activity ($\alpha > 0.5$). Although combinations thereof are allowed in the model, we find it instructive to examine the limiting cases, $\beta = 0$ (perfectly competitive) vs. $\beta = 1$, $\alpha = 1$ (non-competitive).

Considering the perfectly competitive case, with $\beta = 0$ and α arbitrarily set to 1, the mTORC1 activity is given by

$$m = \frac{K_r r}{1 + K_r r + K_p(1-p)}$$

At steady state, it is readily shown that *r* and *p* depend on Akt activity *a* as follows.

$$r = \frac{r_0 a_r^* + a}{a_r^* + a}; \qquad a_r^* = \frac{k_{g0} + k_{-g}(1 + k_{-r}/k_r)}{k_g}$$

$$p = \frac{p_0 a_p^* + a}{a_p^* + a}; \qquad a_p^* = \frac{k_{p0} + k_{-p}}{k_p}$$

Substituting,

$$m = \frac{K_r\left[r_0 a_r^* a_p^* + \left(r_0 a_r^* + a_p^*\right)a + a^2\right]}{\left[1 + K_r r_0 + K_p(1-p_0)\right]a_r^* a_p^* + \left\{(1 + K_r r_0)a_r^* + \left[1 + K_r + K_p(1-p_0)\right]a_p^*\right\}a + (1 + K_r)a^2}$$

This equation shows that mTORC1 activity will respond to that of Akt with substantial ultrasensitivity (with a Hill coefficient approaching 2), provided that certain conditions are met. Evaluating the numerator in the equation above, one condition for ultrasensitivity is

$$r_0 a_r^* + a_p^* \ll 1$$

This inequality is readily achieved, requiring only that the basal Rheb-GTP level, r_0, is low and that PRAS40 phosphorylation is close to saturation; the latter is required for the sensitivity of the neutralization of a negative regulator motif (10). Another condition for ultrasensitivity is based on the denominator of the *m*(*a*) function above:

$$\left[1 + K_r r_0 + K_p(1-p_0)\right]a_r^* a_p^* \gg \left\{(1 + K_r r_0)a_r^* + \left[1 + K_r + K_p(1-p_0)\right]a_p^*\right\}\left(r_0 a_r^* + a_p^*\right)$$

In the limit of r_0 and $p_0 \approx 0$, this inequality reduces to

$$K_p a_r^* \gg \left(1 + K_r + K_p\right) a_p^*$$

This condition is readily met if $K_p \gg 1$ (high affinity of unphosphorylated PRAS40 binding to mTORC1) and $a_r^* \gg a_p^*$ (again, if PRAS40 phosphorylation is highly saturable).

Turning to the noncompetitive case, the mTORC1 activity is given by

$$m = \frac{K_r r}{(1 + K_r r)\left[1 + K_p(1 - p)\right]}$$

Substituting the steady-state expressions for *r* and *p*, and simplifying,

$$m = \frac{K_r\left[r_0 a_r^* a_p^* + \left(r_0 a_r^* + a_p^*\right)a + a^2\right]}{(1 + K_r r_0)\left[1 + K_p(1 - p_0)\right]a_r^* a_p^* + \left\{(1 + K_r r_0)a_r^* + (1 + K_r)\left[1 + K_p(1 - p_0)\right]a_p^*\right\}a + (1 + K_r)a^2}$$

In this case, the numerator inequality is the same, but the denominator inequality (again, in the limit of r_0 and $p_0 \approx 0$) is different:

$$K_p a_r^* \gg (1 + K_r)\left(1 + K_p\right)a_p^*$$

This imposes a constraint on the magnitude of the parameter K_r that is more stringent than the competitive case, but this scenario is nonetheless capable of an ultrasensitive response.

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