Electronic Supplementary Information (ESI)

Exploring higher-order EGFR oligomerisation and phosphorylation – a combined experimental and theoretical approach

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Description of ESI materials:

Table S1 Confidence intervals on parameter estimates

Parameter	Best-fit value[1]	90% Confidence interval[2]
α [3]	5.17×10^{-2}	$4.23\times10^{-2} - 5.24\times10^{-2}$
k_u	6 s^{-1}	$1.28 - 1.18\times10^{4}$ s^{-1}
k_v	1.6 s^{-1}	$6\times10^{-2} - 76.2$ s^{-1}
k_{cx}	15.4 $nM^{-1}s^{-1}$	5.29 – 65.5 $nM^{-1}s^{-1}$
k_{cr}	8.89 s^{-1}	0.66 – 12.31 s^{-1}
χ	4.37×10^{4} nM	$1.07\times10^{4} - 3.9\times10^{5}$ nM

[1]The best-fit parameter values are the nominal parameter values (Table 1) used in simulations.
[2]Confidence intervals were estimated by bootstrapping. The bootstrapping procedure used is that described by Press et al. (2007). The synthetic datasets used in the bootstrapping procedure were derived from the data presented in Fig. 4A.
[3]The parameter α is a constant factor used to relate the predicted cluster density to the normalized fluorescence data of Fig. 4A.

Citation: Press WH, Teukolsky SA, Vetterling WT, Flannery BP (2007) *Numerical Recipes: the Art of Scientific Computing*, 3rd Edition. Cambridge University Press.

Table S2 Parameter sensitivity

Parameter	Sensitivity coefficients[1] for amount of receptor tetramer	Parameter	Sensitivity coefficients[1] for level of receptor phosphorylation
RT	1.06	RT	1.06
k_{cr}	-4.99×10^{-02}	k_{p}	4.98×10^{-01}
k_{cx}	4.99×10^{-02}	k_{dp}	-4.98×10^{-01}
λ_{f2}	2.98×10^{-02}	k_{cx}	4.95×10^{-02}
λ_{r2}	-2.96×10^{-02}	k_{cr}	-4.93×10^{-02}
χ	2.53×10^{-02}	λ_{f2}	2.89×10^{-02}
k_{v}	-2.23×10^{-02}	λ_{r2}	-2.88×10^{-02}
k_{u}	2.15×10^{-02}	χ	2.45×10^{-02}
λ_{f1}	5.04×10^{-03}	k_{v}	-2.22×10^{-02}
λ_{r1}	-4.92×10^{-03}	k_{u}	2.14×10^{-02}
$k_{r,1}$	4.51×10^{-03}	λ_{f1}	4.91×10^{-03}
$k_{f,3}$	4.45×10^{-03}	λ_{r1}	-4.77×10^{-03}
$k_{f,1}$	-4.12×10^{-03}	$k_{r,1}$	4.38×10^{-03}
$k_{r,3}$	-3.61×10^{-03}	$k_{f,3}$	4.34×10^{-03}
$k_{r,2}$	-3.61×10^{-03}	$k_{f,1}$	-3.99×10^{-03}
LT	3.38×10^{-04}	$k_{r,3}$	-3.51×10^{-03}
$k_{f,2}$	2.38×10^{-04}	$k_{r,2}$	-3.51×10^{-03}
λ_{f0}	2.21×10^{-04}	LT	3.56×10^{-04}
k_{p}	1.21×10^{-04}	$k_{f,2}$	2.37×10^{-04}
k_{dp}	1.21×10^{-04}	λ_{f0}	2.20×10^{-04}
λ_{r0}	9.57×10^{-05}	λ_{r0}	9.63×10^{-05}

[1]Sensitivity coefficients are defined as $\frac{x_i}{y_j}\left(\frac{\partial y_j}{\partial x_i}\right)$, where x_i represents the value of a model parameter and y_j represents the steady-state value of a model variable, either the amount of EGFR tetramer or the level of EGFR phosphorylation. The partial derivative in each sensitivity coefficient is calculated via a finite-difference approximation: $\frac{\partial y_j}{\partial x_i} \sim \frac{\Delta y_j}{\Delta x_i}$, where Δx_i represents a 1% change in the nominal value of parameter x_i (Table 1) and Δy_j represents the resulting change in the steady-state value of the variable y_j. A positive (negative) sensitivity coefficient indicates that an increase in the value of the corresponding parameter value causes an increase (decrease) in the value of the corresponding variable. The sensitivity coefficients given here characterize the robustness of the nominal steady state at a ligand concentration of 30 nM.

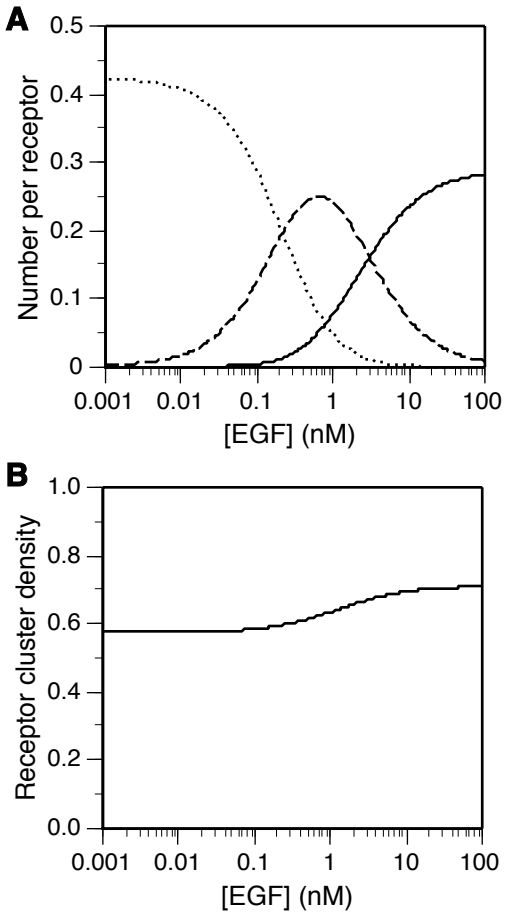

Fig. S1 Predictions of a dimer-only model. This model corresponds to the reaction scheme illustrated in Box I of Fig. 1 and is equivalent to a model studied by Macdonald and Pike (2008) and others. (A) The predicted levels of preformed EGFR dimers (D_0/R_T, dotted line), dimers bound to a single ligand (D_1/R_T, broken line), and dimers bound to two ligands (D_2/R_T, solid line) are shown as a function of ligand (EGF) dose. If we assume that receptor phosphorylation correlates with the level of dimers bound to two ligands, then the ligand dose-dependence of receptor phosphorylation is similar to that observed experimentally (compare the solid line in this panel to the data shown in Fig. 2D). (B) Receptor cluster density calculated as $(R+B+D_0+D_1+D_2)/R_T$ as a function of ligand dose (cf. Fig. 4A). As can be seen, receptor cluster density is predicted to increase with increasing ligand dose, which is a known property of the model considered here that has previously been discussed in the literature (Macdonald and Pike, 2008; Macdonald-Obermann and Pike, 2009). The results shown here can be obtained from the model of Macdonald and Pike (2008), which has four equilibrium binding constants (L_{20}, K_{11}, K_{21}, and K_{22}) that can be specified independently. The values of these parameters are as follows: $L_{20}=\lambda_{f0}/\lambda_{r0}=212$ nM^{-1}, $K_{11}=k_{f,1}/k_{r,1}=4.6$ nM^{-1}, $K_{21}=k_{f,2}/k_{r,2}=5.3$ nM^{-1}, and $K_{22}=k_{f,3}/k_{r,3}=0.34$ nM^{-1} (cf. Table 1). In our experiments, and in the calculations performed to obtain the results shown here, the total receptor concentration is 0.09 nM (90,000 EGFR per cell and 6×10^5 cells/ml) (cf. Table 1). Here, R, B, D_0, D_1, D_2, and R_T represent the concentrations of free monomeric receptors, bound monomeric receptors, ligand-free dimers, singly-bound dimers, doubly-bound dimers, and all receptors, respectively.

Citations:

Macdonald JL, Pike LJ (2008) Heterogeneity in EGF-binding affinities arises from negative cooperativity in an aggregating system. *Proc. Natl. Acad. Sci. USA* **105**:112-117.

Macdonald-Obermann JL, Pike LJ (2009) The intracellular juxtamembrane domain of the epidermal growth factor (EGF) receptor is responsible for the allosteric regulation of EGF binding. *J. Biol. Chem.* **284**:13570-13576.

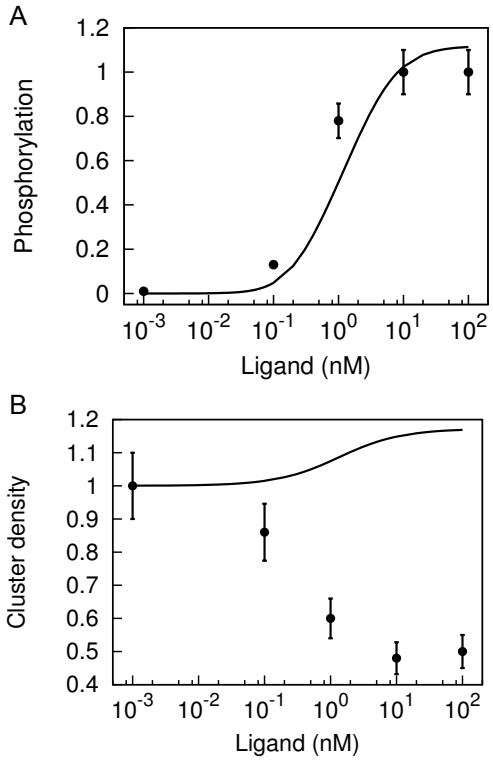

Fig. S2 Predictions of a linear oligomers-only model. This model is a special case of the model of Fig. 1 for which χ=0 (i.e., the case where the ring-closure reactions, which are parameterized by the rate constants λ_f and k_{cx}, in Box VI of Fig. 1 do not occur). (A) Receptor phosphorylation as a function of ligand dose (cf. Fig. 5). (B) Receptor cluster density as a function of ligand dose (cf. Fig. 4A).

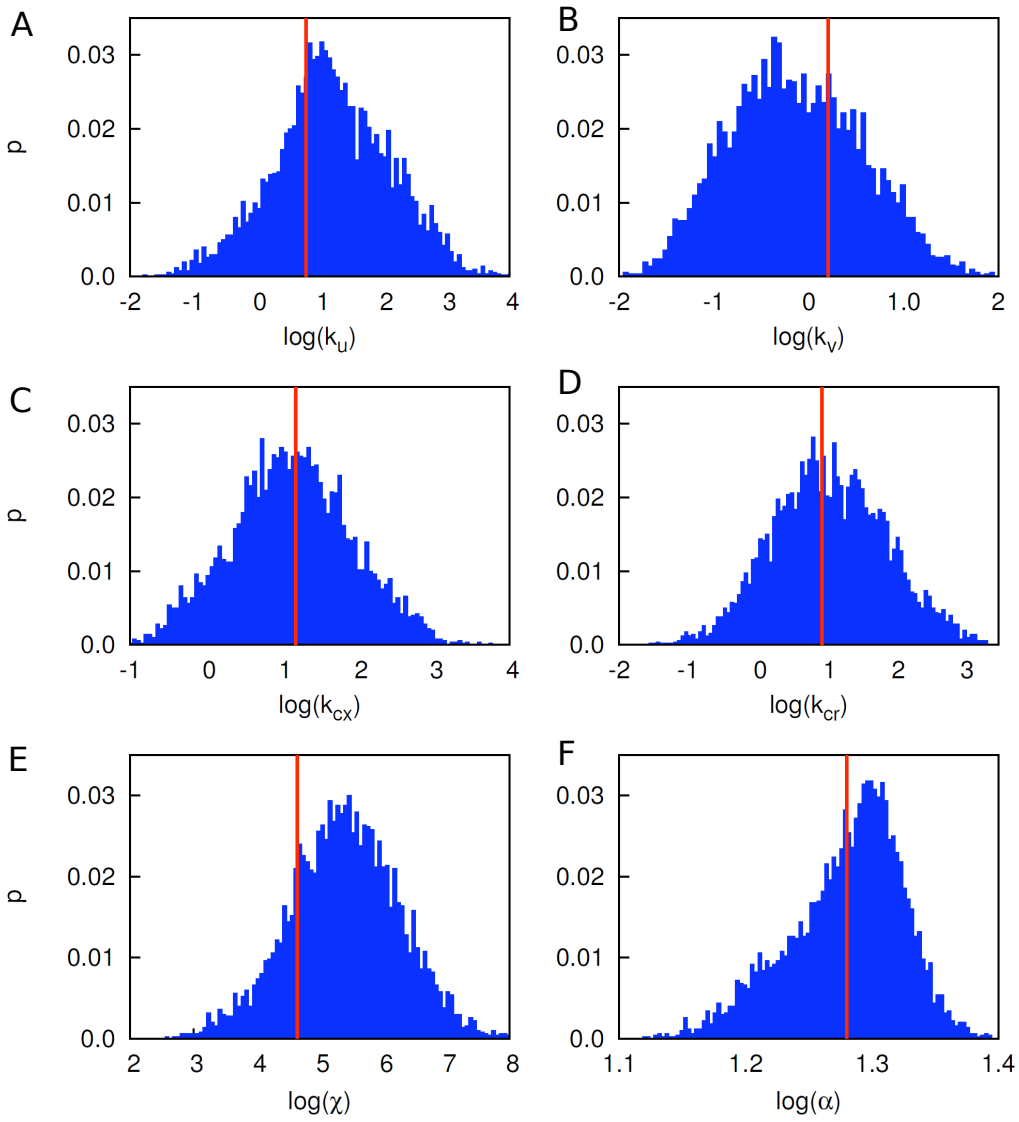

Fig. S3 Marginal posterior distributions for each of the six adjustable parameters of the model of Fig. 1: (A) k_u (B) k_v (C) k_{cx} (D) k_{cr} (E) χ and (F) α. Each panel presents a histogram obtained via the Bayesian parameter estimation procedure described in Appendix S1. Note that the horizontal axis is logarithmic (base 10). In each panel, the red vertical line corresponds to the best-fit value listed in Table 1.

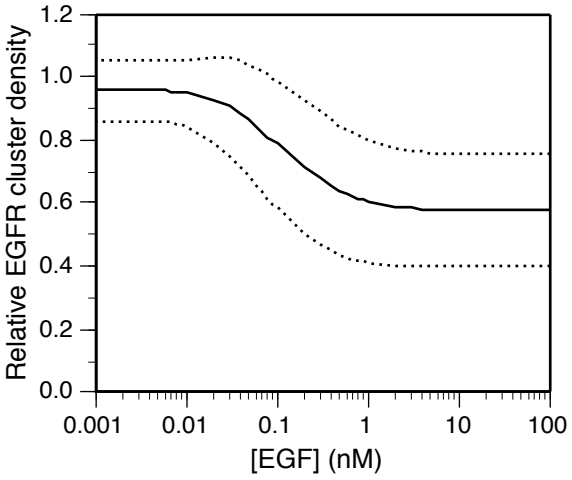

Fig. S4 Robustness of model behavior. The curve of Fig. 4A was calculated 100 times as before (i.e., by simulation of the model of Fig. 1 to steady state for different ligand doses over the range indicated). However, in each of the 100 cases, the six adjustable model parameter values used were not those of Table 1 but rather a particular set of values sampled from the joint posterior distribution found via Bayesian parameter estimation (Appendix S1). The solid curve passes through the averages obtained at the various ligand doses from the 100 calculations. Each point along one of the two dotted curves corresponds to an average plus or minus the standard deviation. As can be seen, receptor cluster density is robustly predicted to decrease with increasing ligand dose. This model behavior is qualitatively distinct from the behaviors of the reduced models considered in Figs. S1 and S2.