Hypothesis

" The model assumes that phosphorylation of Shc leads to a significant reduction cant reduction in its affinity for EGFR, which is primarily responsible for the predicted damping of the initial response to EGF" [1]

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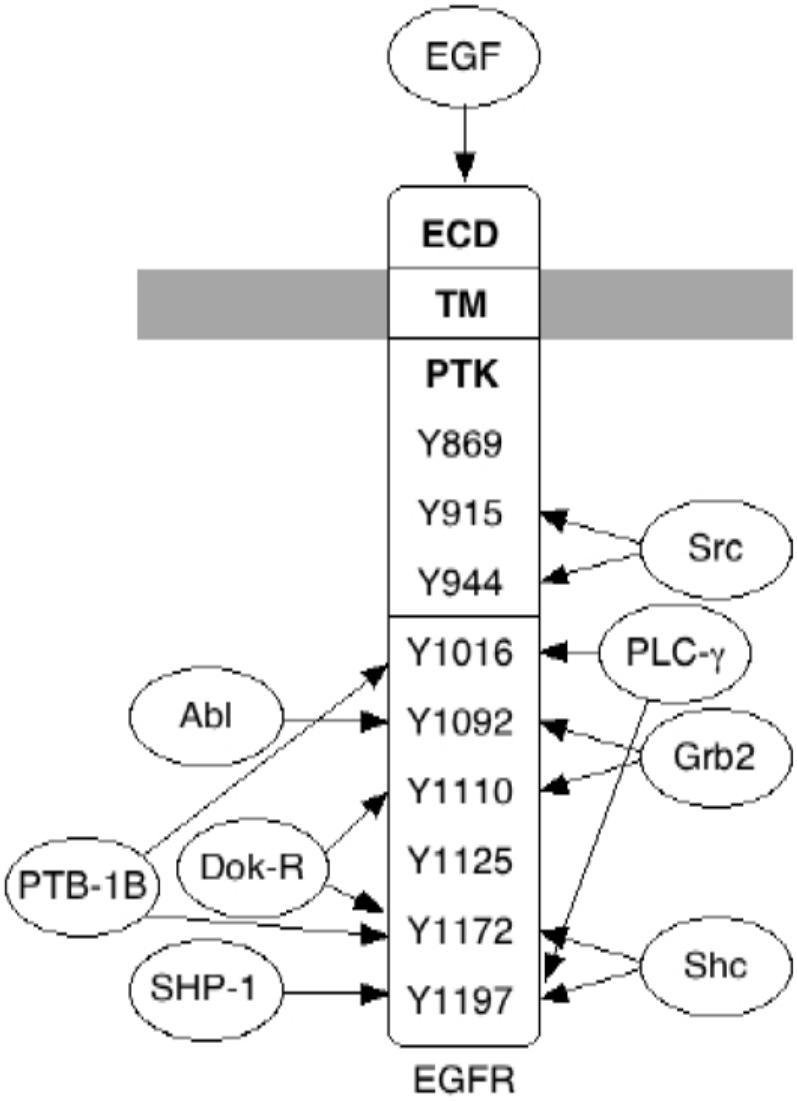
& Truong Ngo

Multiplicity of sites and binding partners gives rise to combinatorial complexity



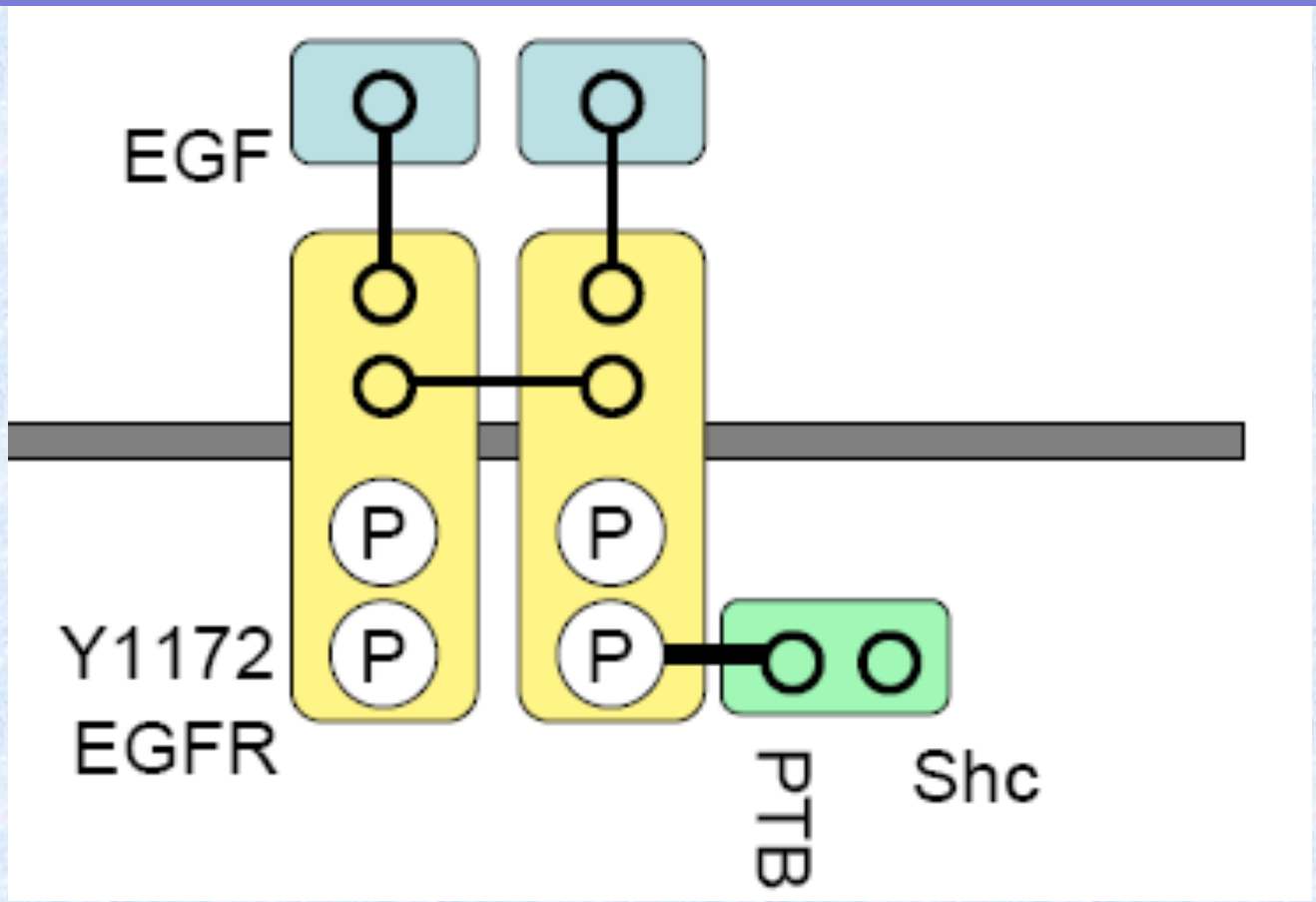
Epidermal Growth Factor Receptor (EGFR)

- 9 sites $\longrightarrow$ 2^9 phosphorylation states
- Each site has ≥ 1 binding partner $\longrightarrow$ more than 3^9 total states
- EGFR must form dimers to become active $\longrightarrow$ more than 1.9×10^8 states

Shc Pathway

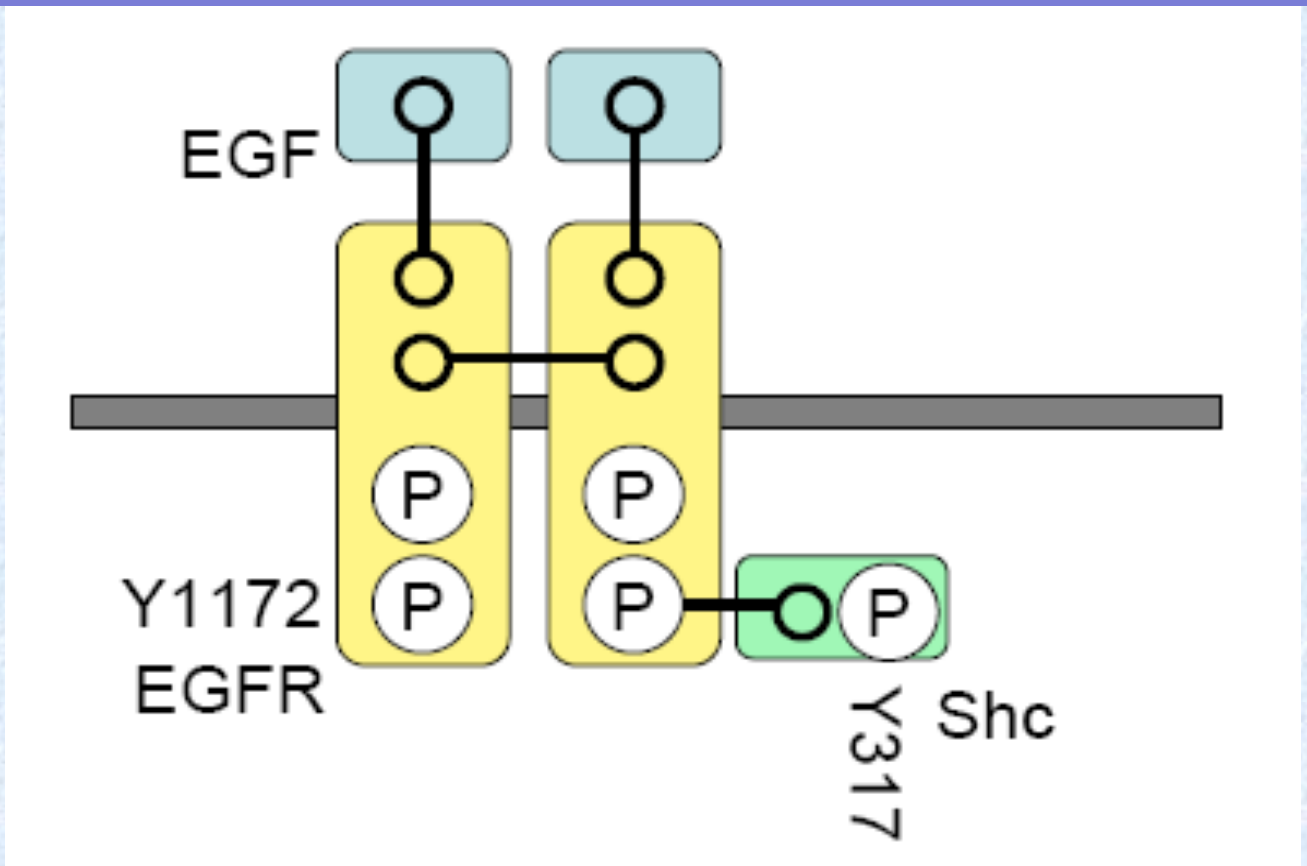
1. EGF binds EGFR
2. EGFR dimerizes
3. EGFR transphosphorylates itself
4. Shc bind phospho-EGFR
5. EGFR transphosphorylates Shc
6. Grb2 binds phospho-Shc
7. Sos binds Grb2 (activationpath)

Shc Pathway



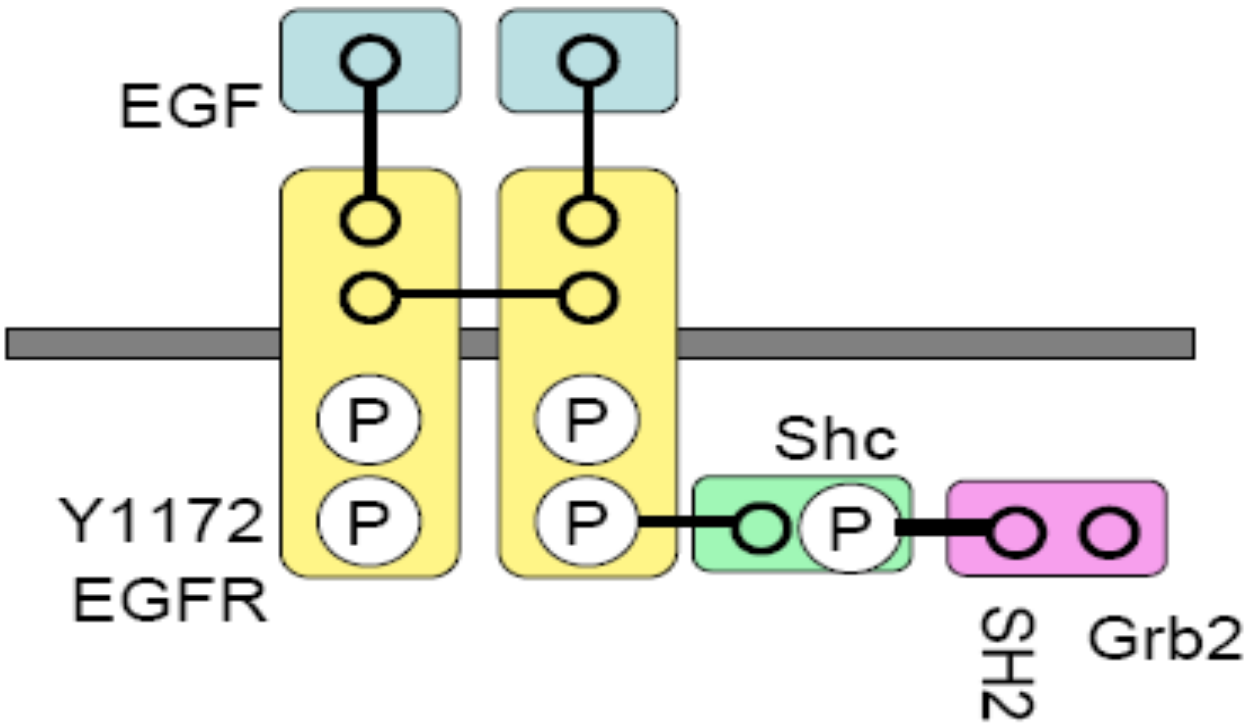
Shc bind phospho-EGFR

Shc Pathway



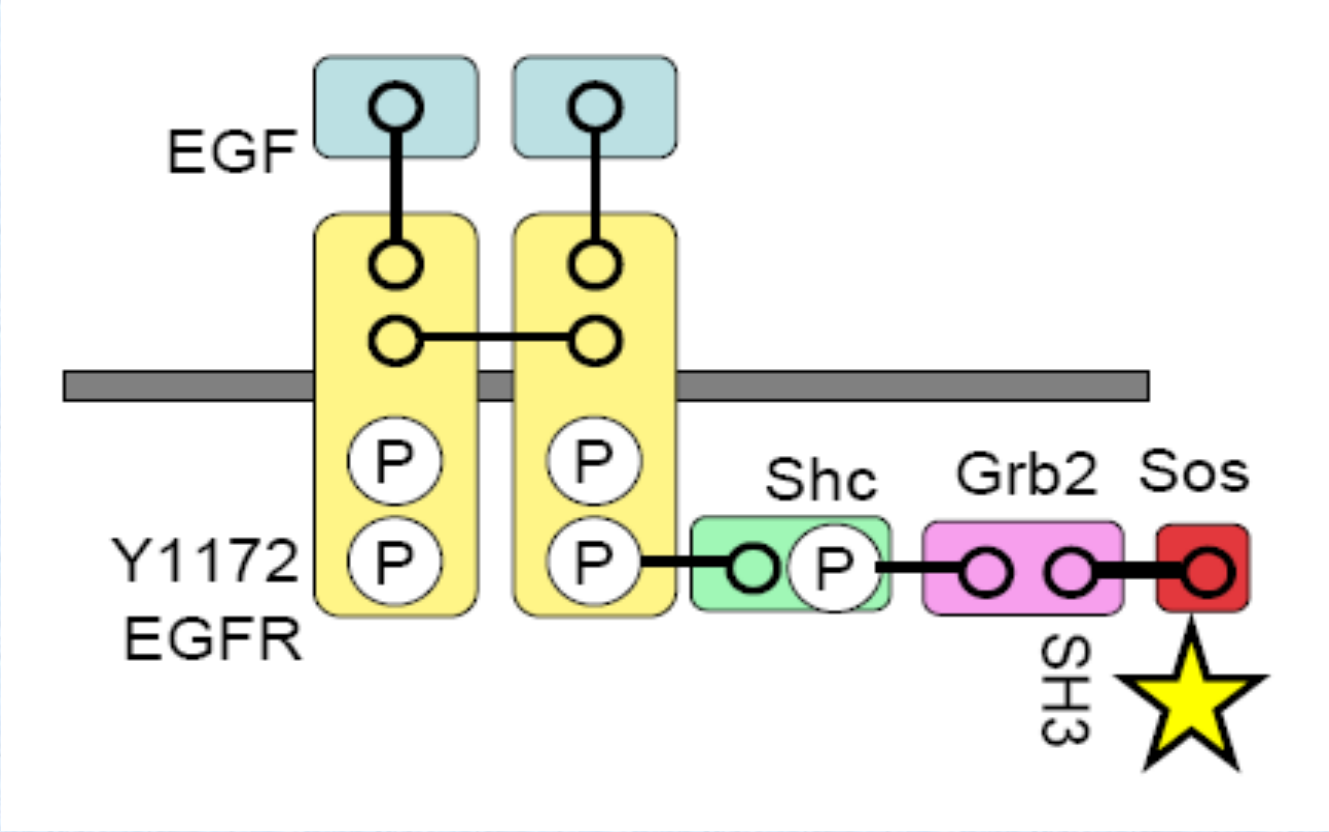
EGFR tansphosphorylates Shc

Shc Pathway



Grb2 binds phospho-Shc

Shc Pathway



Sos binds Grb2 (activationpath)

Facts

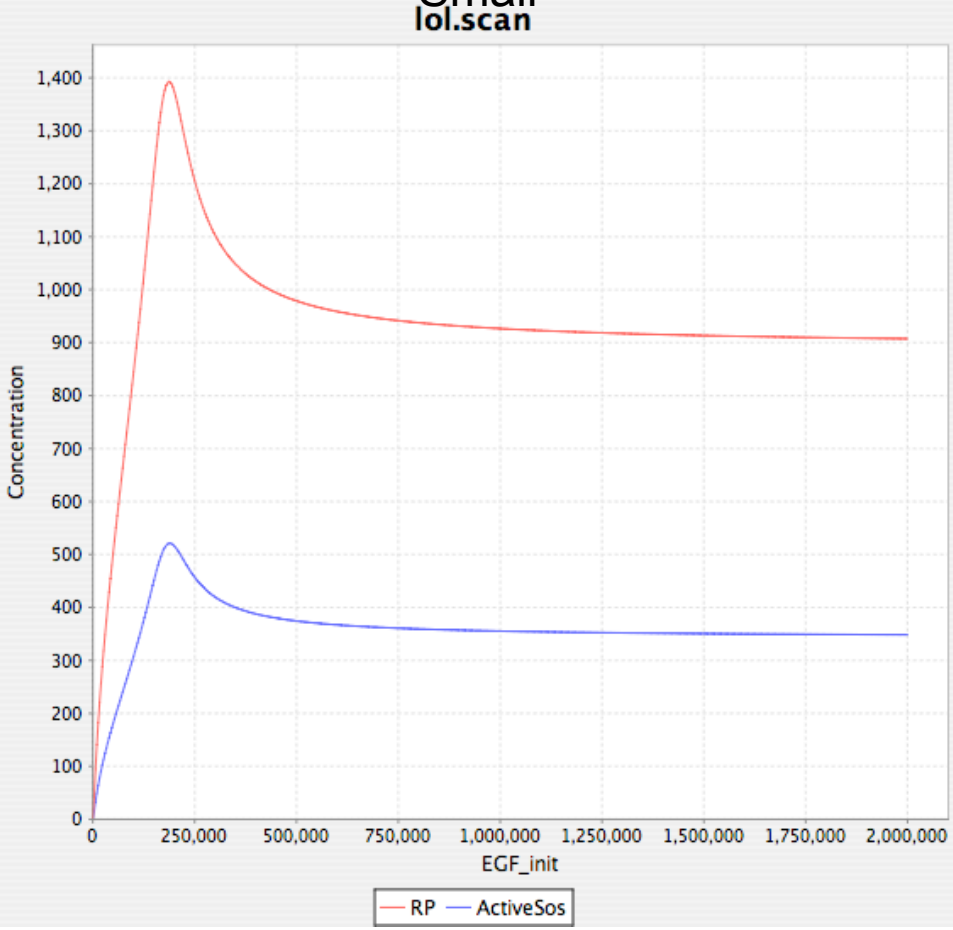
- Three protein that directly interact with phosphotyrosine residues on the receptor : Gb2; Shc and PLCγ.
- We also assume that when Grb2, Shc or PLCγ are bound to EFGR, the corresponding phosphotyrosine residues are not available to receptor phosphotyrosinephosphatases.
- It has been reported that the Grb2-Sos complex binds to both EGFR- and Shc- derived phosphopeptides with higher affinity than Grb2 alone.

Varying initial levels of EGF, and the responses or the various species (time 600)Observables

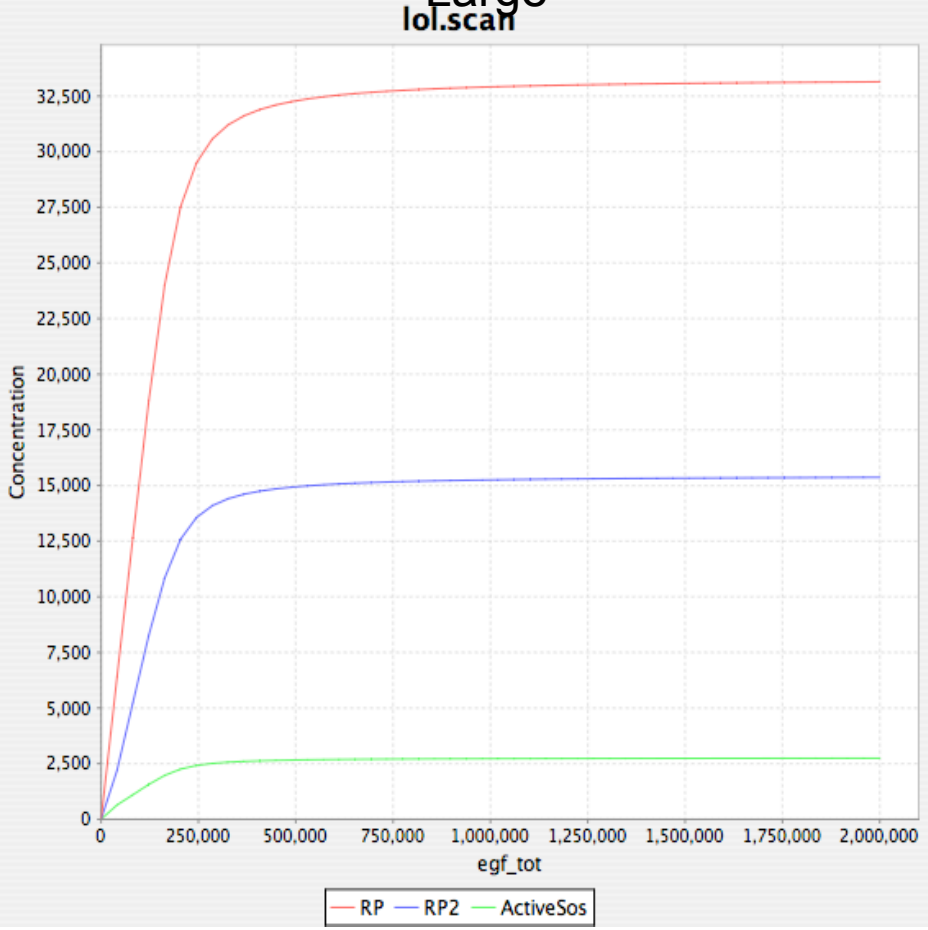
RP: EGFR(Y1068~P!?)
ActiveSos : Sos1.EGFR

RP : egfr(Y1068~pY!?)egfr(Y1148~pY!?)
RP2 : egfr(Y1068~pY!?)
ActiveSos : Sos.egfr

Small



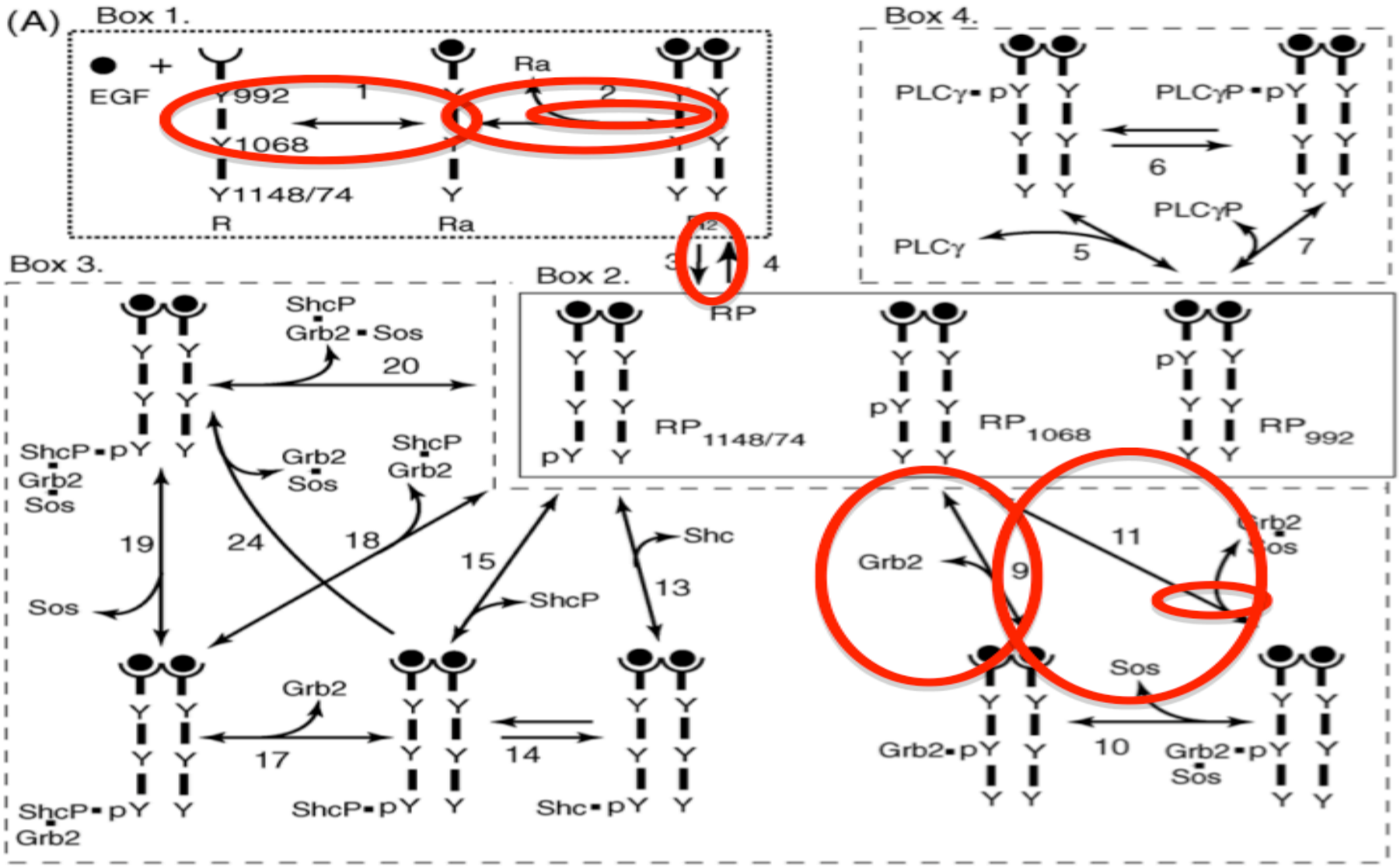
Large



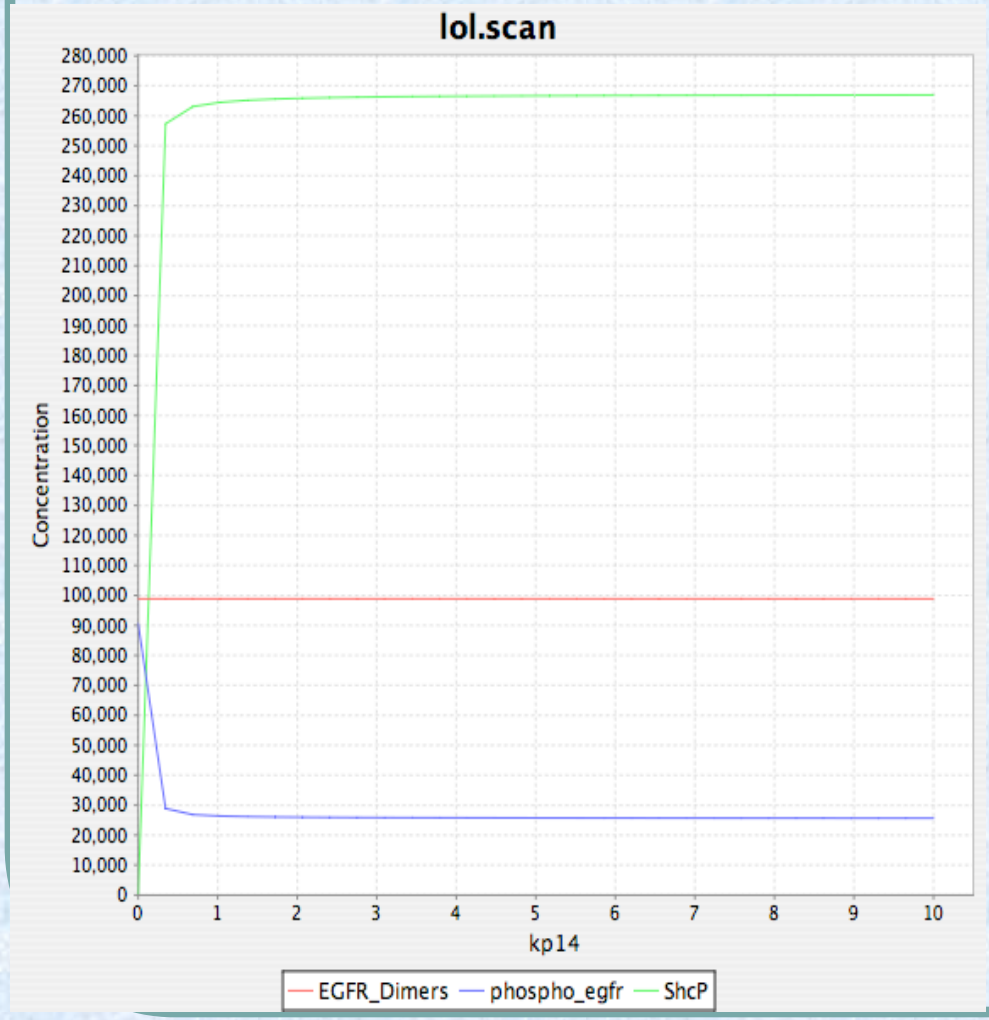
Ambiguity

1) Does Kholendenko's hypothesis concern the initial amount of EGF, or is s/he referring to the activeSos produced?
2) We decided the latter makes more sense…
3) So, we looked at rates of ShcP production, and compared to activeSos levels.

Reaction13, 14 & 15: ShcP/Shc levels & rates



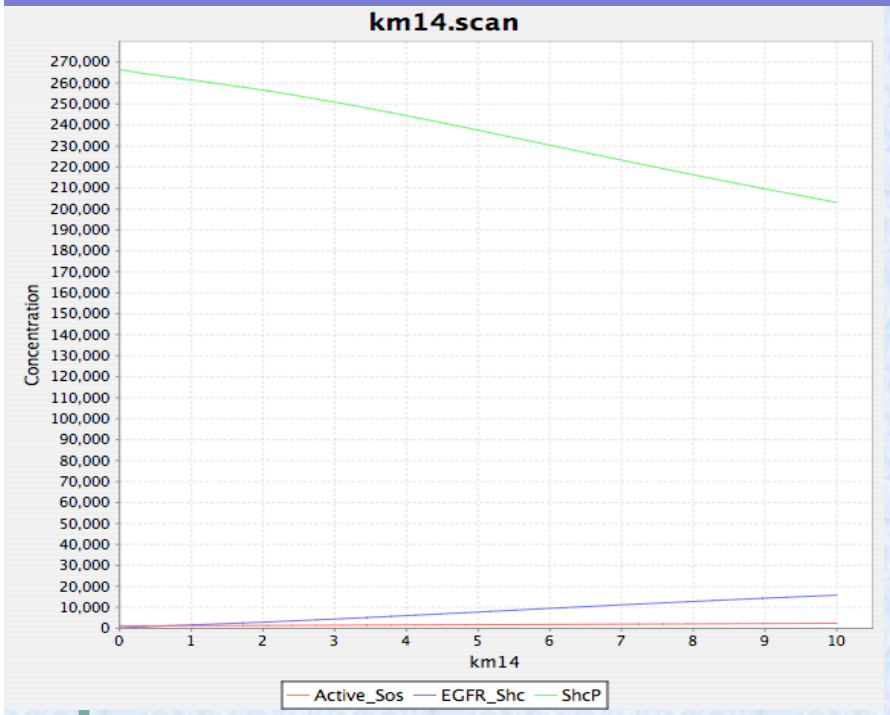
Initial change of kp 14

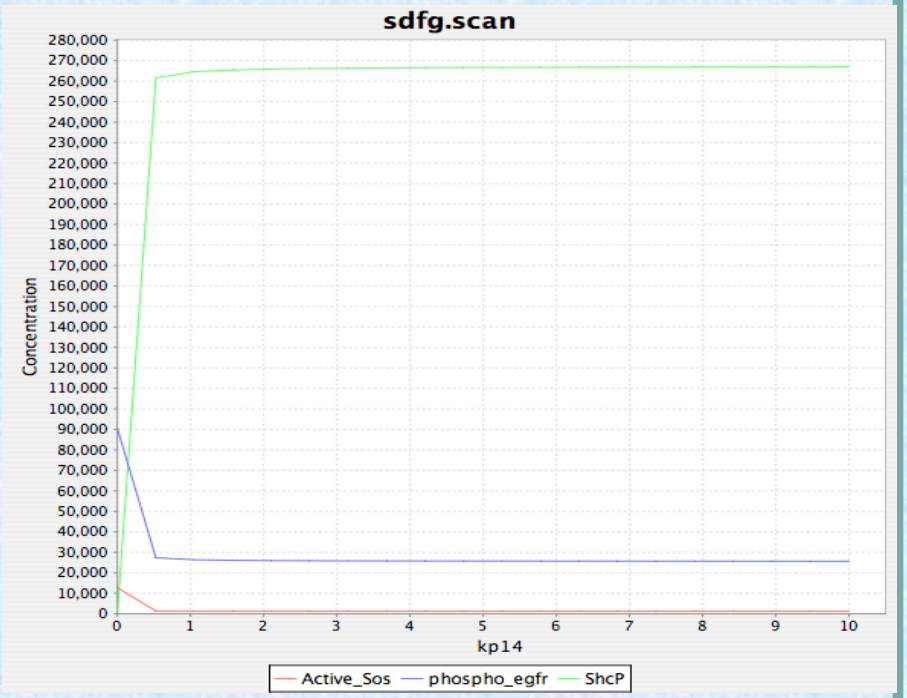


1. egfr(r!2,Y1148~pY!1).Shc(PTB!1,Y317~Y) -> egfr(r!2,Y1148~pY!1).Shc(PTB!1,Y317~pY) kp14
2. We initially thought the change of the numbers of ShcP would effect the graph of receptor dimers (based on 1st interpretation of Kholendekno). However, we could not see any change in the dimer graph that verified this theory.

(we used the network model for these tests)

Kp14 , km 14

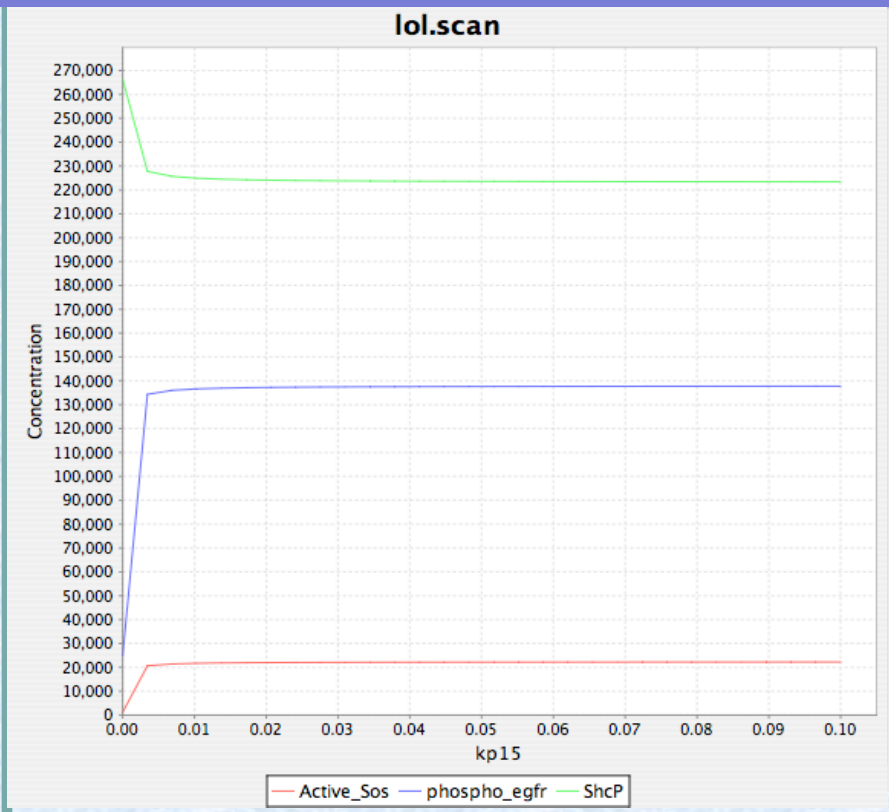


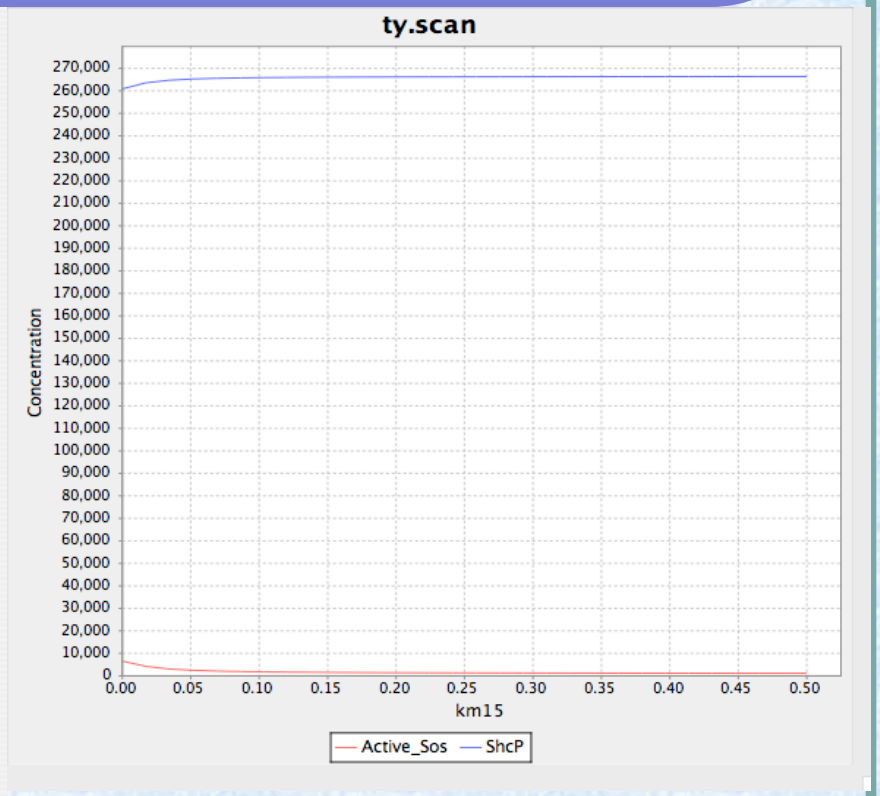


1. egfr(r!2,Y1148~pY!1).Shc(PTB!1,Y317~Y) -> egfr(r!2,Y1148~pY!1).Shc(PTB!1,Y317~pY) kp14
2. Shc(PTB!1,Y317~pY) -> Shc(PTB!1,Y317~Y) km14

3. We increased the rate of the production of ShcP (kp14). It resulted in decreased number of active Sos. (plateau?)
4. We also plotted an increase of km14, which by this theory, should result in more activatedSos (which it does).

kp 15, km15



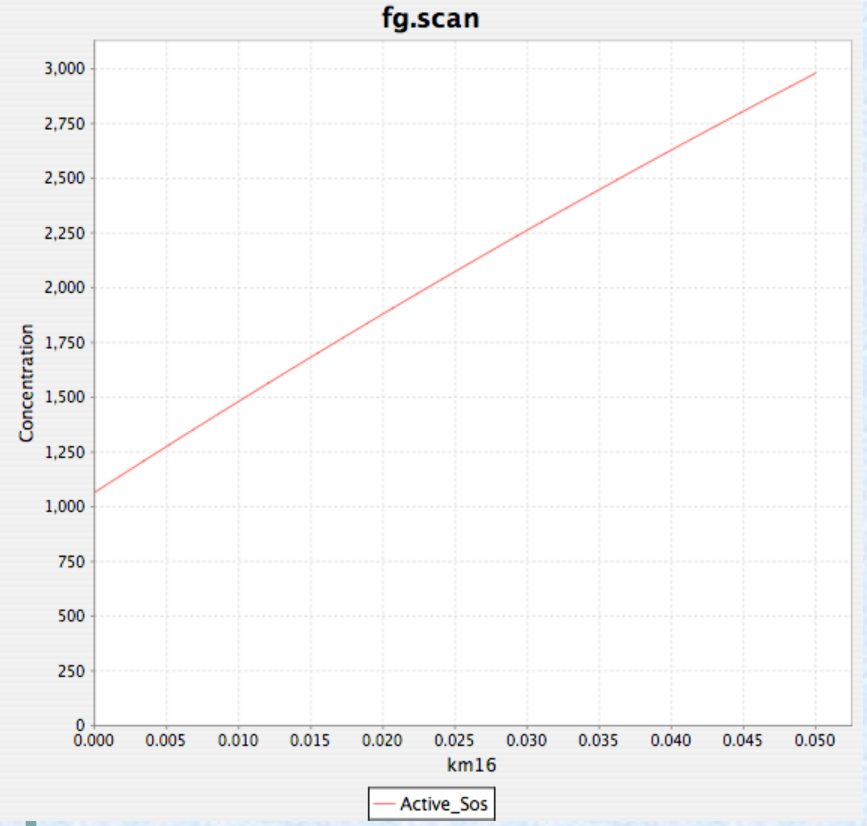


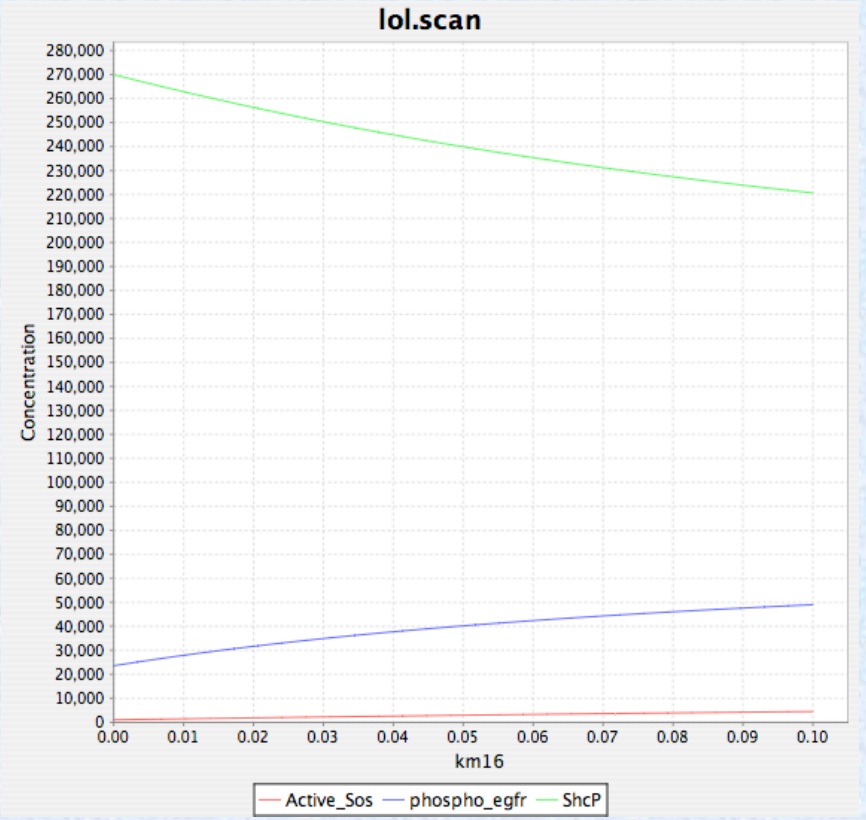
egfr(Y1148~pY) + Shc(PTB,Y317~pY) <-> egfr(Y1148~pY!1).Shc(PTB!1,Y317~pY) kp15,km15

(time = 100 sec)

The amount of ShcP and activeSos is inversely related initially, but both plateau with more than a minimal rate in either direction

Km 16

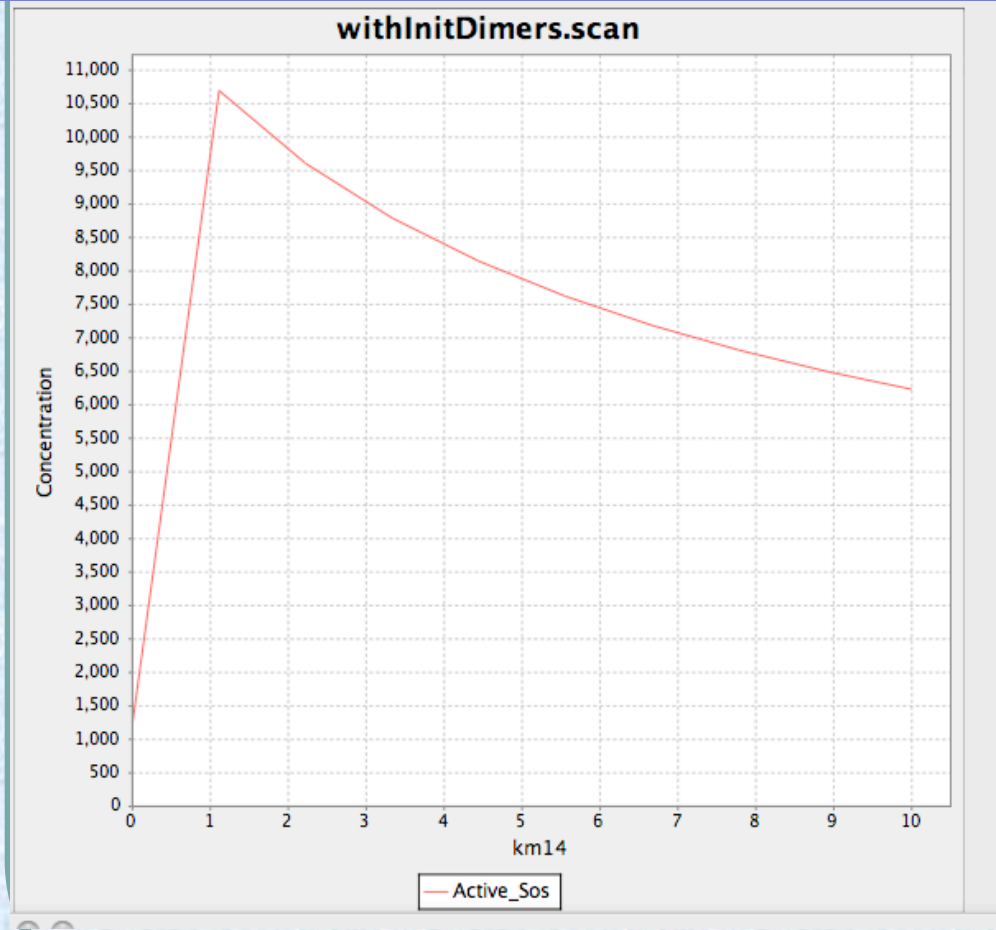




Shc(PTB,Y317~pY) -> Shc(PTB,Y317~Y) km16

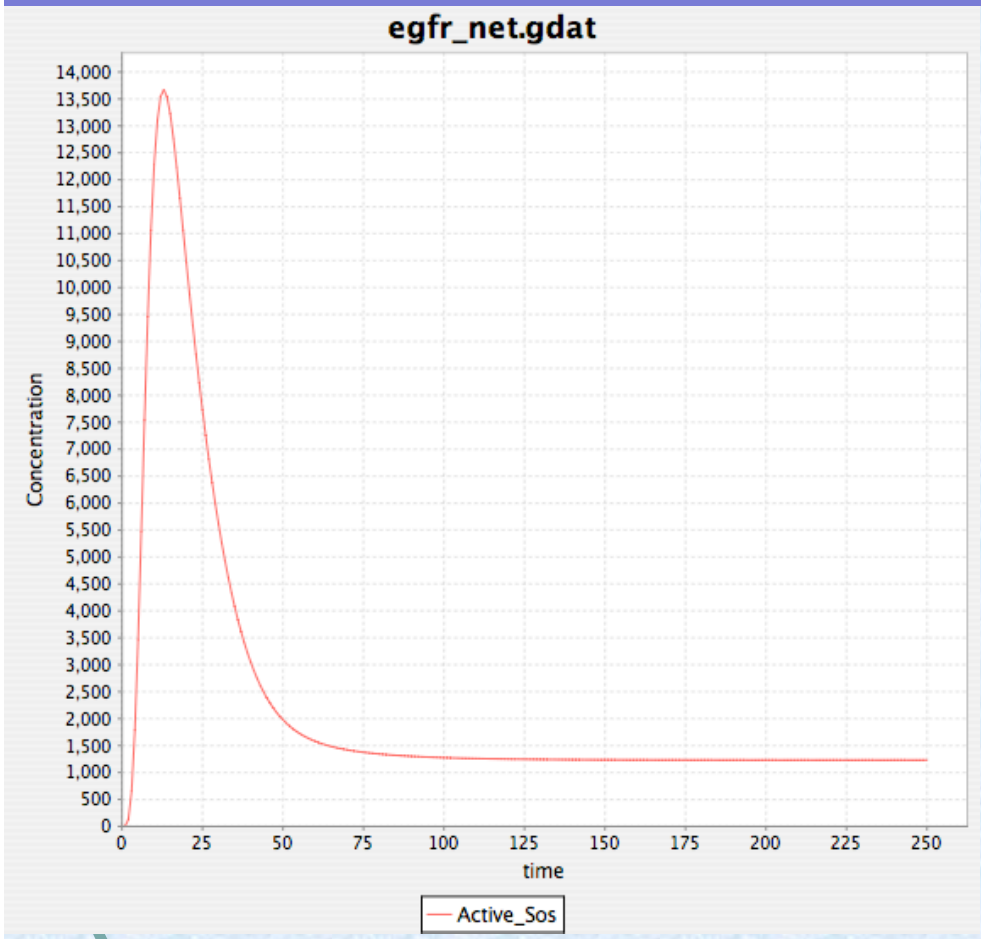
Cytosolic reaction (not involving receptors) Direct relation here : rate of km16 (Shc dephosphorylized) increases the level of activeSos

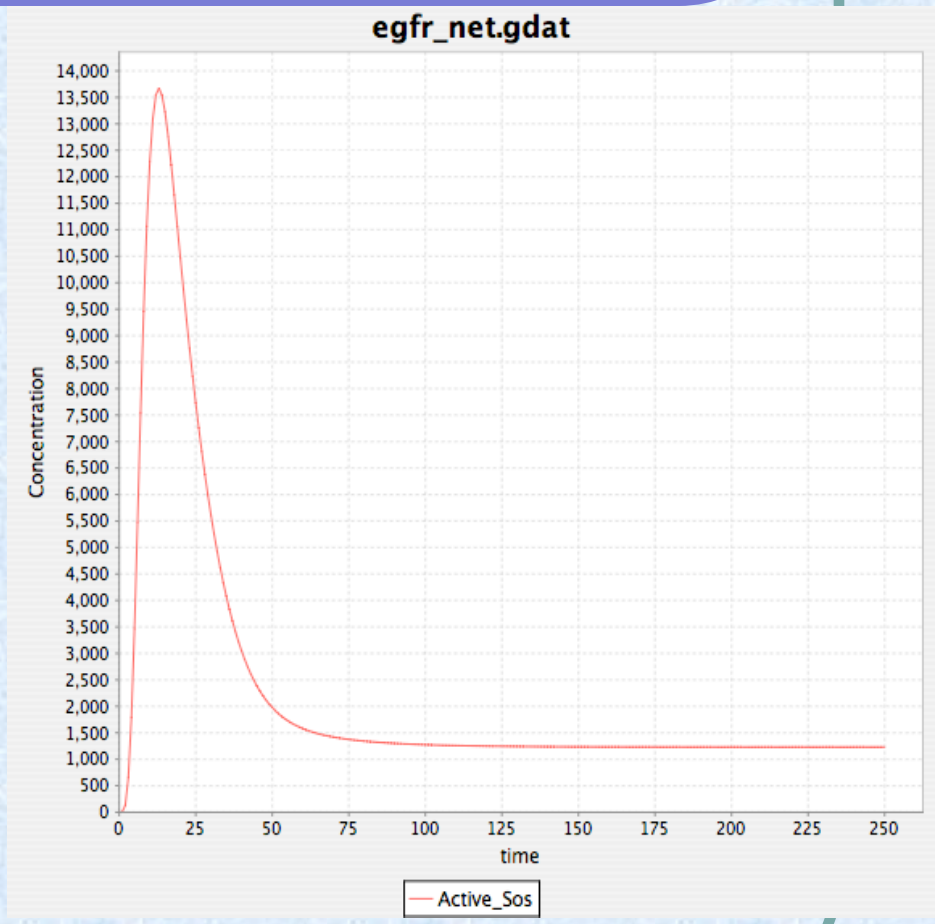
We tried to simulate the addition of “preformed dimers” as mentioned by Jorissen, as a factor that may complicate the relation of ShcP to signaling



Km14 rate w/ a low added amount of preformed Dimers (time 10 seconds)…
ActiveSos now decreases as km14 increases (Shc->ShcP)…supports Kholodekno’s theory?)
But what about initial increase of activeSos seen here?
Possible conclusion : Kholodenko’s theory holds, within constraints (InitDimer=20 gives similar graph)

Compare activeSos with and without preformed dimers





activeSos with preformed dimers and activeSos without preformed dimers are the same

Future Directions

- **We have described the model of EGFR systems. This model have been used to analyze the system and to predict new and unexpected properties.**

So what have we learned and how should this guide our future efforts in systems biology?

Future Directions

First, we have learned that models are only useful if experiments can be designed that directly test specific predictions of the model; in other words, the predictions must be in a form that can be directly measured in an experiment. A model that predicts an increase in receptor dimerization, for example, is only useful if we can actually measure that parameter. The slow progress in building comprehensive models of the EGFR system is primarily due to the difficulty of experimental design and execution. A model that takes only a few weeks to construct might take years or even decades to test adequately.[2]

Future Directions

Second, we have found that as models become more complex, the amount of data necessary to validate them becomes greater than can be generated by usual laboratory experiments. This experimental constraint has tended to keep the models small. So, we have models of trafficking, signaling, heterodimerization and so forth, but it will be very difficult to combine these into larger, integrated models until we can determine the best experimental way to validate such complex constructs. This is where the new, high-throughput data-generation approach espoused by systems biology promises to have the greatest impact. Computer-based models can be used as 'high-throughput hypotheses' to exploit these high-throughput data-generation techniques, such as advanced imaging or mass-spectrometry-based proteomics.[2]

Future Directions

- **Finally,** the ultimate test of any model is whether it can provide a useful higher-level perspective of a complex problem. From this viewpoint, we believe the EGFR models have been a resounding success. For example, we have learned that receptor trafficking controls the information flux through cells. Cell-surface signaling represents the instantaneous information presented to the cell, whereas endosomal signaling represents the integration of information over many cycles of cell-surface binding. We have found that ligand availability is the master regulator of the EGFR system and that regulated ligand proteolysis controls virtually all downstream receptor activities.[2]

Class Discussion

Some aspects of the model of Kholodenko et al. (1999) are controversial and might be subject to future investigation and refinement. For example, the model assumes that phosphorylation of Shc leads to a significant reduction in its affinity for EGFR, which is primarily responsible for the predicted damping of the initial response to EGF. Although recent molecular dynamics simulations support a lower affinity of phosphorylated Shc for EGFR (Suenaga et al., 2004), the implication that Shc recruitment and phosphorylation negatively regulates signaling is problematic in light of earlier experimental work on EGFR signaling (Sasaoka et al., 1994). In addition, pre-formed dimers of EGFR (Jorissen et al., 2003) and other complicating features of ligand-induced receptor dimerization that may influence signaling (Wofsy et al., 1992; Klein et al., 2004) are omitted in the model of Kholodenko et al. (1999) and its extensions. Our main focus here, however, is to evaluate the effects of simplifying assumptions made in developing the pathway-like model, and we therefore keep both the basic reaction processes and their accompanying rate constants in the network model so that we can make a controlled comparison of the two models.

References

1. Michael L. Blinov, James R. Faeder, Byron Goldstein and Willian S. Hlavacek " A network model of early events in epidermal growth factor receptor signaling that accounts for combinatorial complexity" , Biosystems, vol 83, 2006, pp.136-151
2. http://www.sciencedirect.com/science?_ob=ArticleURL&_udi=B6TCX-4778D05-2&_user=699449&_rdoc=1&_fmt=&_orig=search&_sort=d&_docanchor=&view=c&_acct=C000039260&_version=1&_urlVersion=0&_userid=699449&md5=5f5a6278789265 8e1f81ab0477e9b8ba
3. http://www.csml.org/models/csml-models/egfr-pathway/