Sensitivity analysis

In order to find the right set of parameters and to evaluate de accuracy of the model. We made a sensitivity analysis where we evaluated the how the response of the system depends on each parameter.

1. kuo: Michaelis Menten Constant for the phoshporylation LuxU-LuxO.

When CAI-I is not present in the media the phoshporylation LuxU-LuxO has a higher rate than the phosphase reaction. If the parameter is high then there is more LuxO phosphorylated when CAI-I arrives, and in consequence the system taker longer to response as can be seen in Figure 1

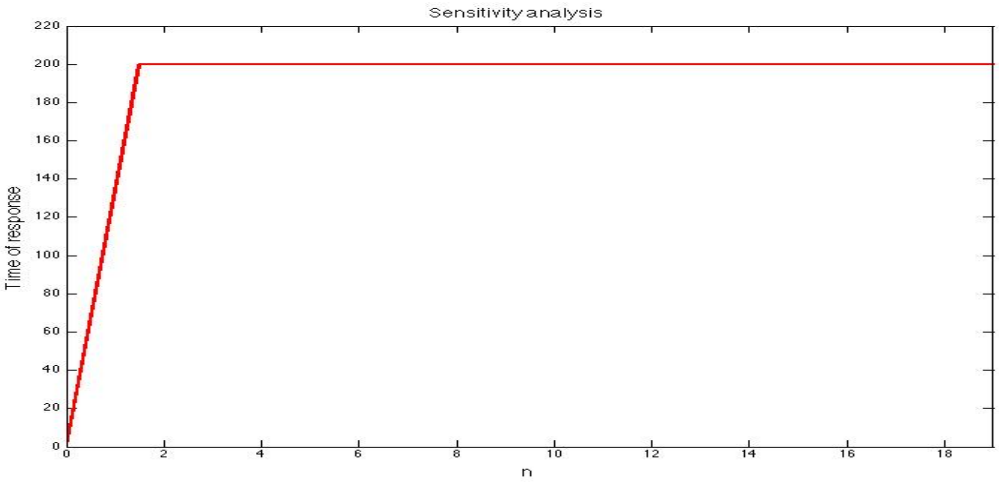

Figure 1.1 Time of response when kuo varies

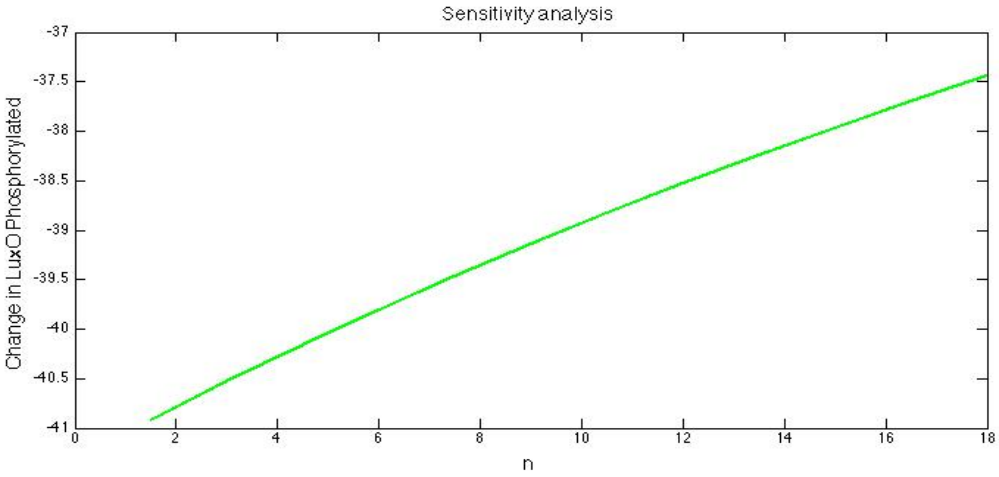

Figure 1.2 Change in LuxO phosphorilation vs kuo

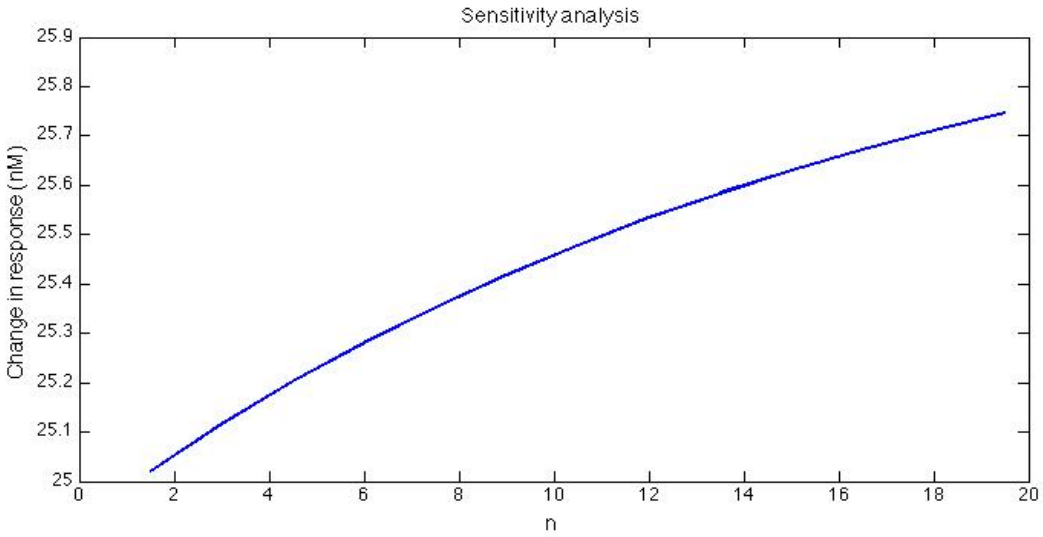

Figure 1.3 Change in response vs kuo

2. CS basal production rate.

While there is no CAI in the culture media, the CqsS production will increase constantly because it is under a constitutive promoter. Once CAI is present the dephosphorylation cascade, if the numbers of CqsS are high, the time of response of the system will be longer and the change in the response less; because CqsS will be inactive and therefore inhibiting the dephosphrylation cascade.

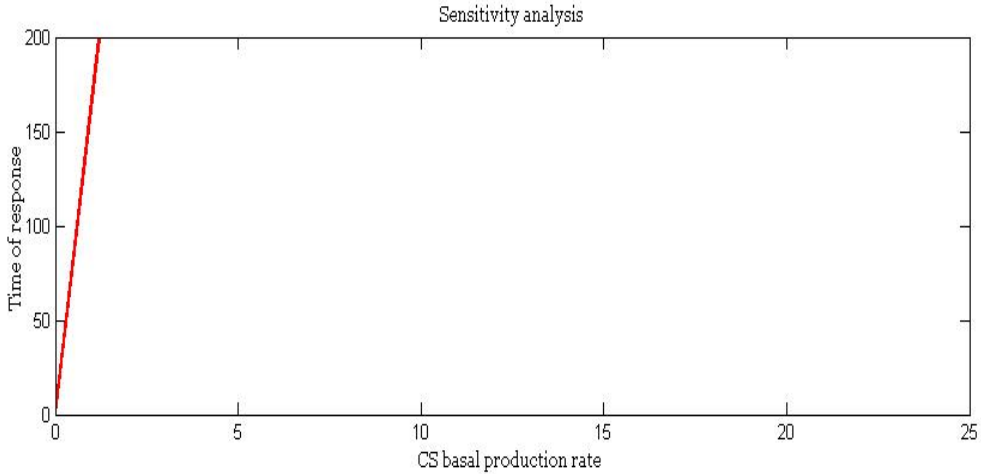

Figure 2.1 Time of response when CS basal production rate varies

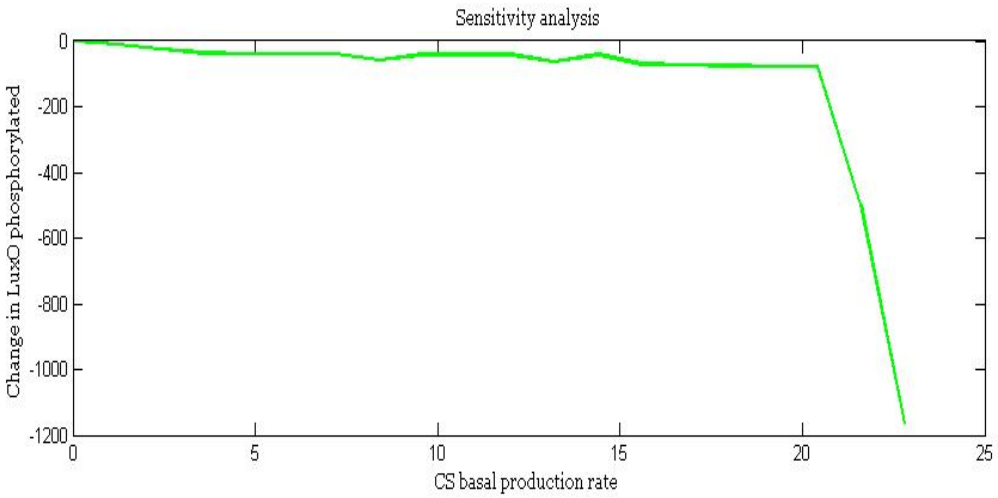

Figure 2.2 Change in LuxP phosphorylated when CS varies

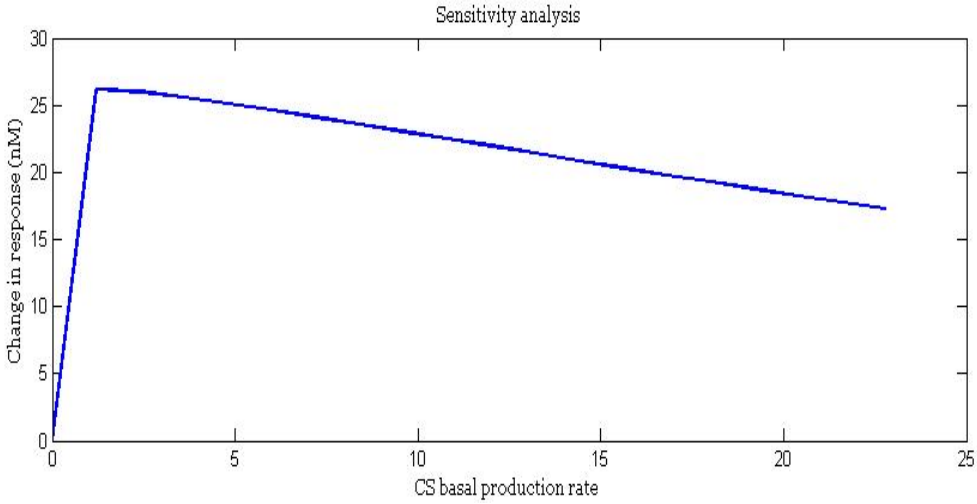

Figure 2.3 Change in response when CS varies

3. TA basal production rate.

TA is not directly involved in the phosphorylation of LuxO, and consequently no there is no visible change as the parameter value increases when compared to LuxO (Figure 3.1). On the other hand the time of response will decrease as the TA basal production increases, however if it increases too much the change in response will not be as significant, because the response might even be triggered even without the presence of CAI .

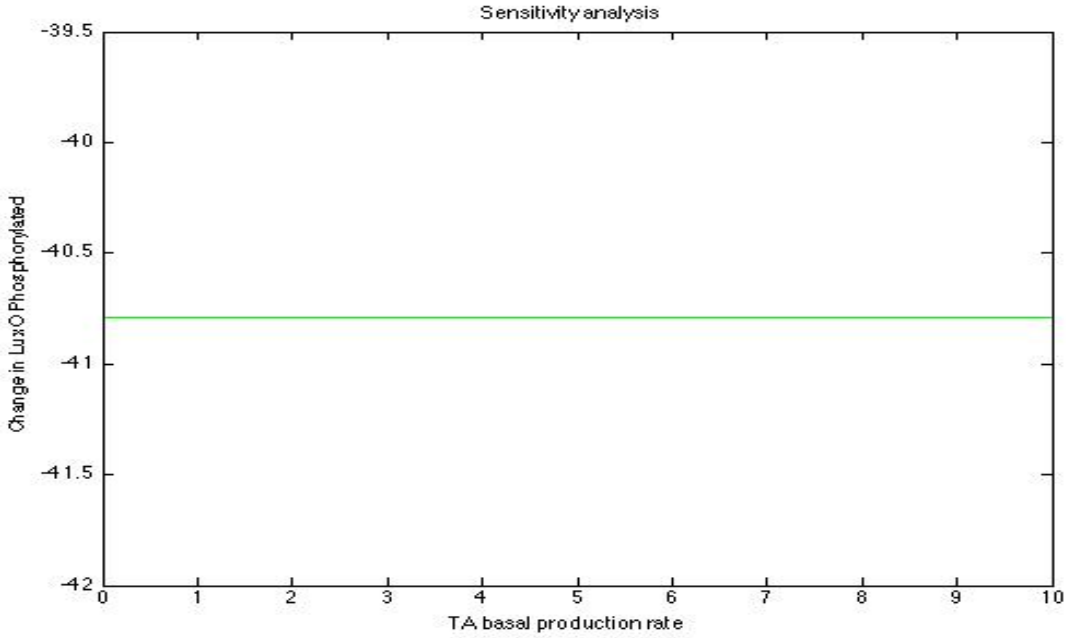

Figure 3.1 Change in LuxO phosphorylated when ata varies

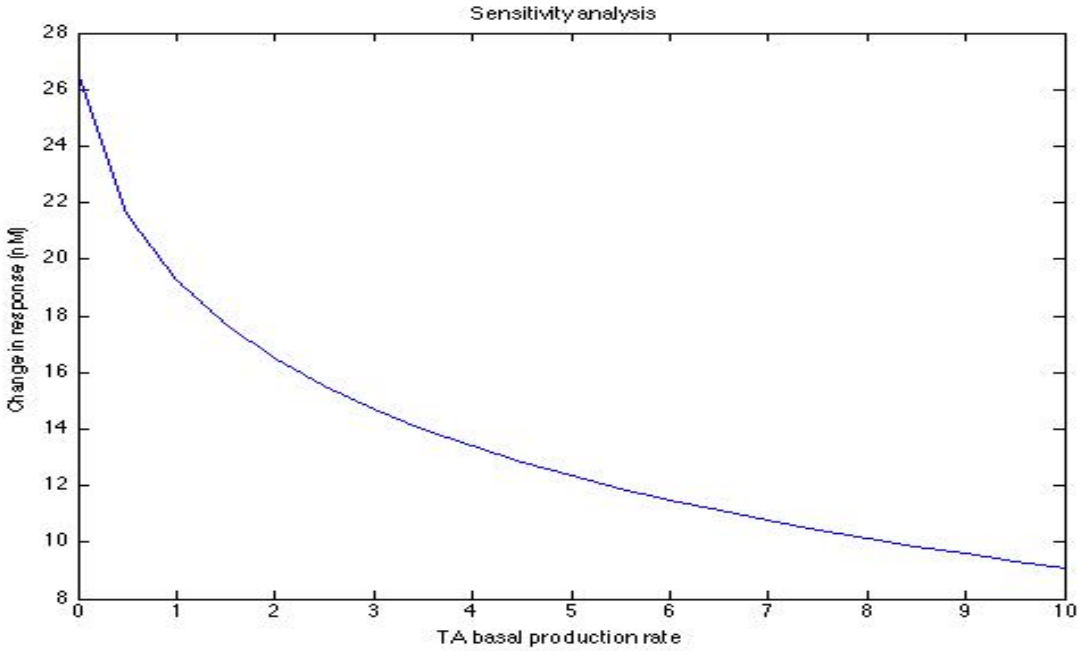

Figure 3.2 Change in response when ata varies

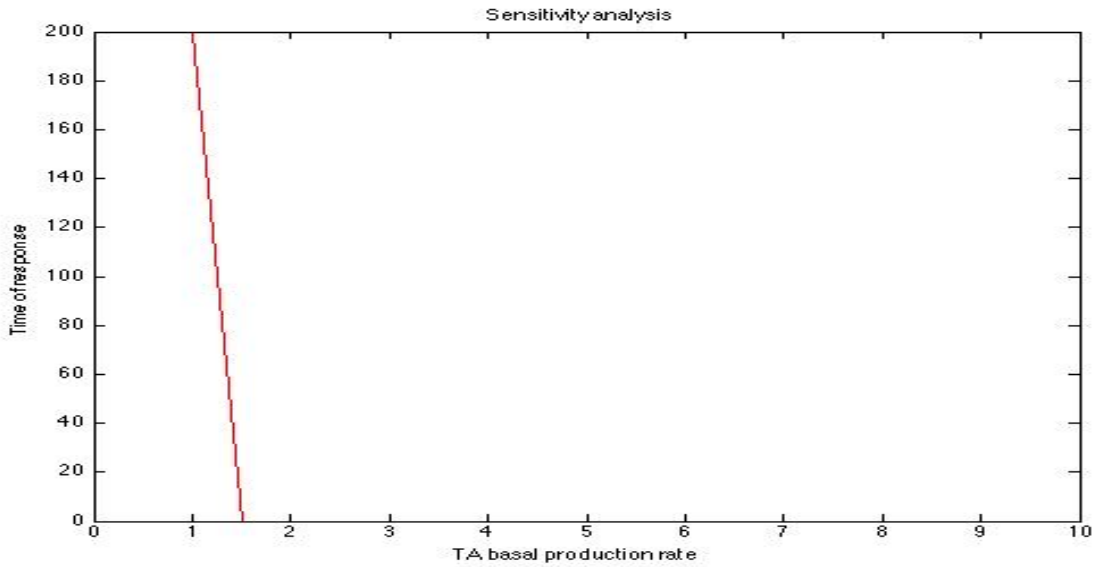

Figure 3.3 Change in time when ata varies

4. TR basal production rate

The time of response, unlike the previous parameter is constant and independent of this parameter. When the Basal production rate of the repressor is too high, the change in response is too low, this is because even in the presence of CAI there will be enough repressor to interfere with the production of the response and even when there is no phosphorylated LuxO the repressor will still be being produced.

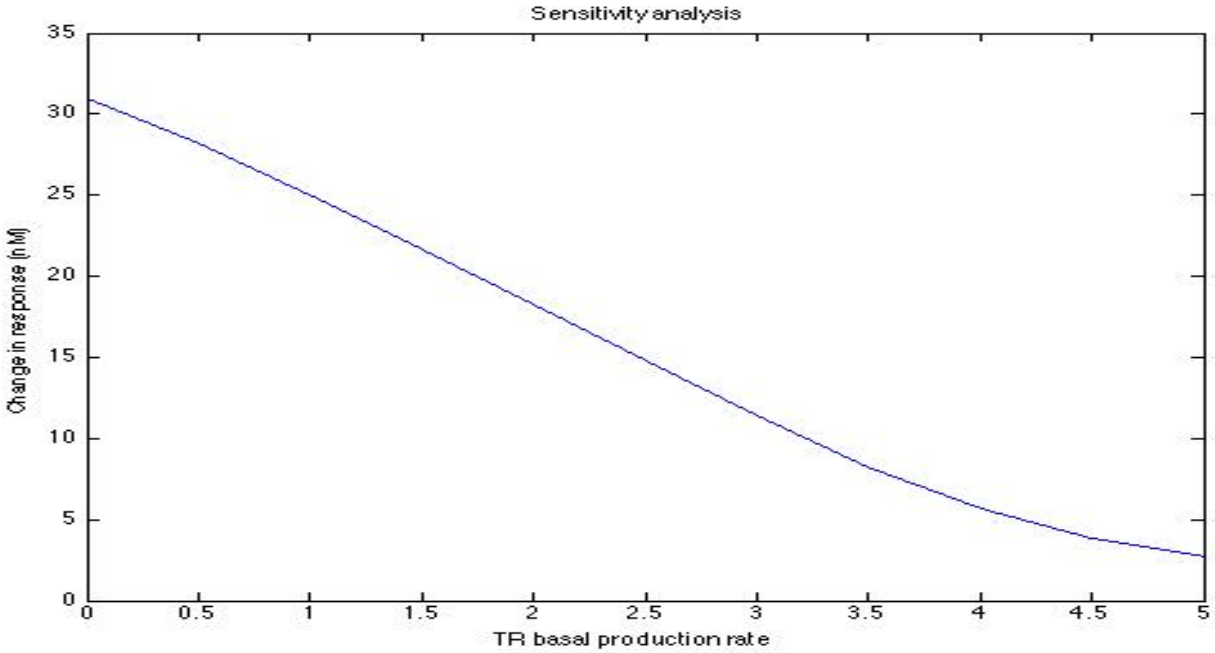

Figure 4.1 Change in response when atr varies

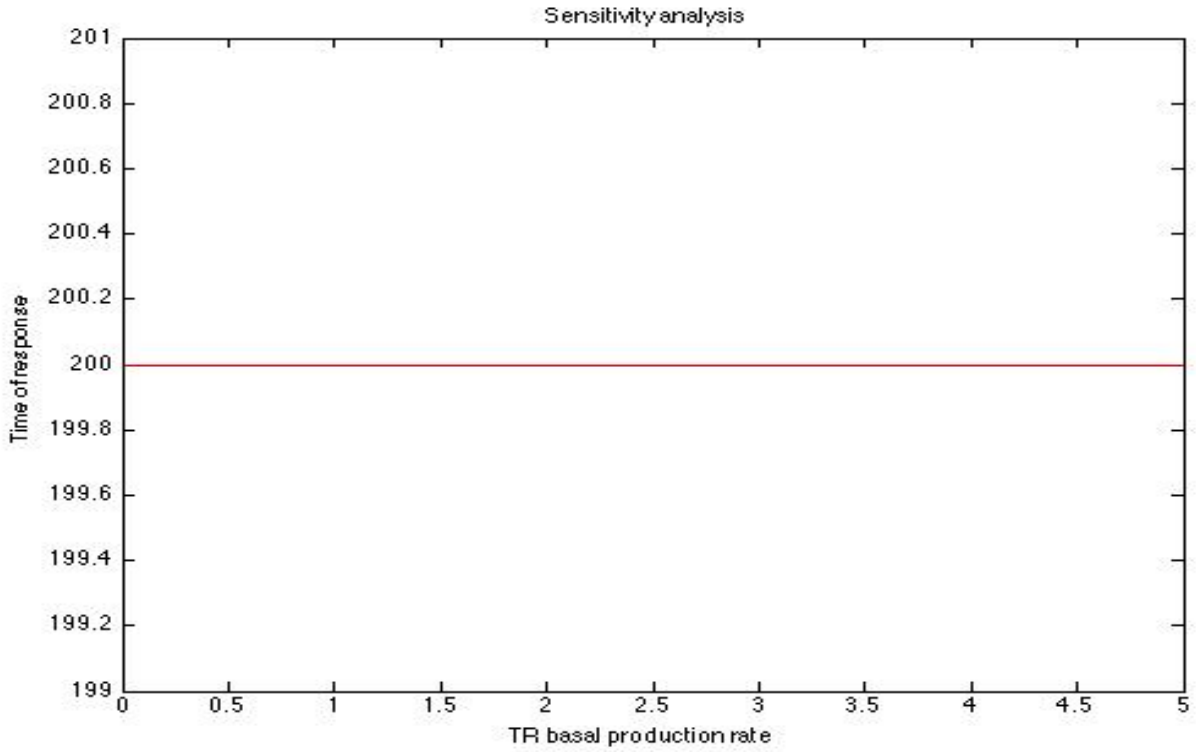

Figure 4.2 Change in time response when atr varies

5. LuxU basal production rate

The system's response is very sensitive to this parameter. Whenever LuxU levels are high all LuxO can be dephosphorylated and make the response's slope much steeper. However when the parameter assumes high values eventually raises dramatically the amount in phosphorylated LuxO (figura 5.2) and the response goes down (figura 5.3), which indicates that the dephosphorylation capacity of the cell reaches a maximum and the opposite process starts to dominate in the cell, making this a not desirable response. This parameter requires careful choosing.

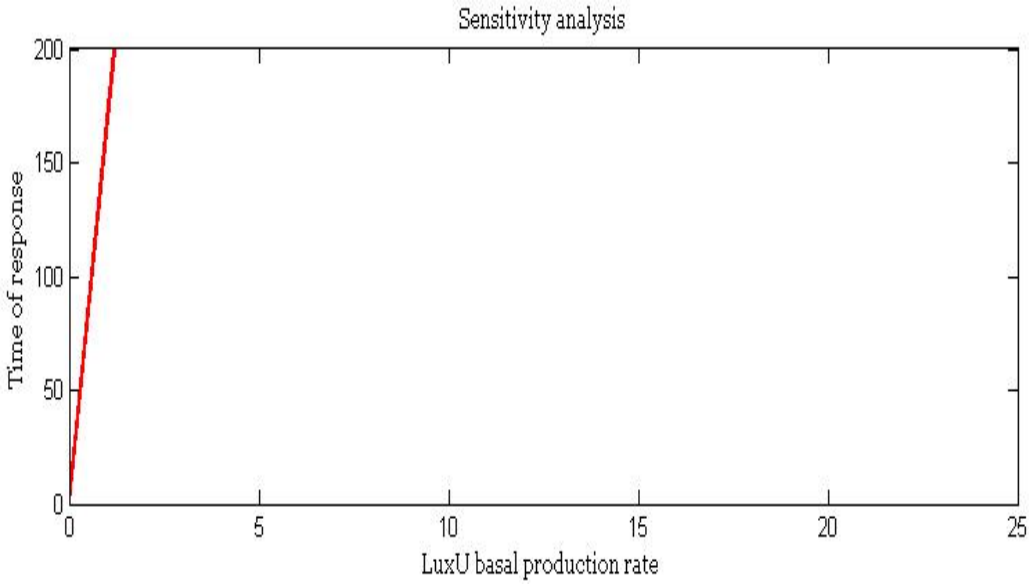

Figure 5.1 Change in time response when au varies

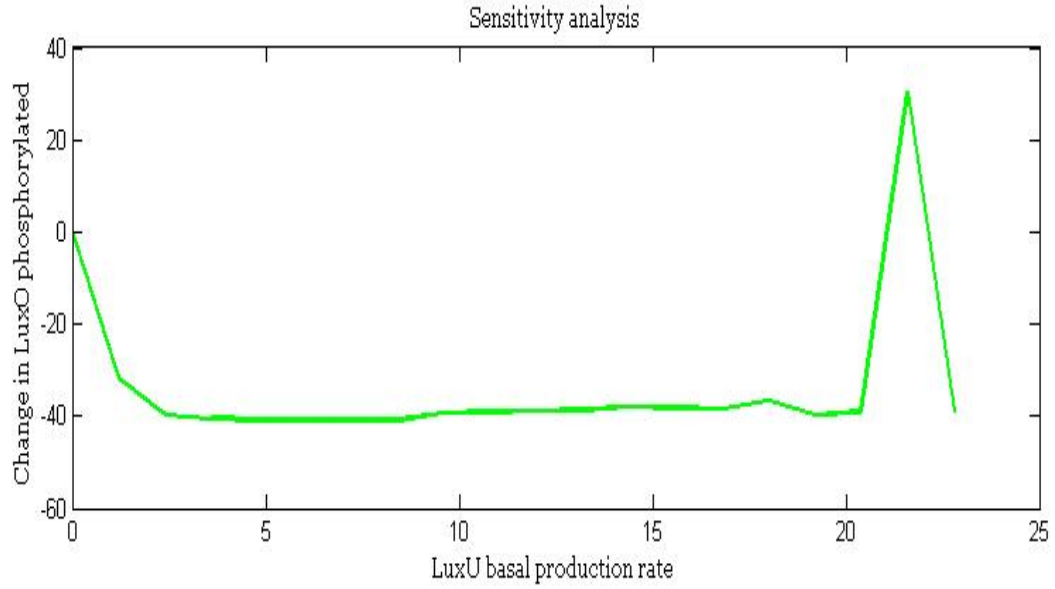

Figure 5.2 Change in LuxO phosphorylated when au varies

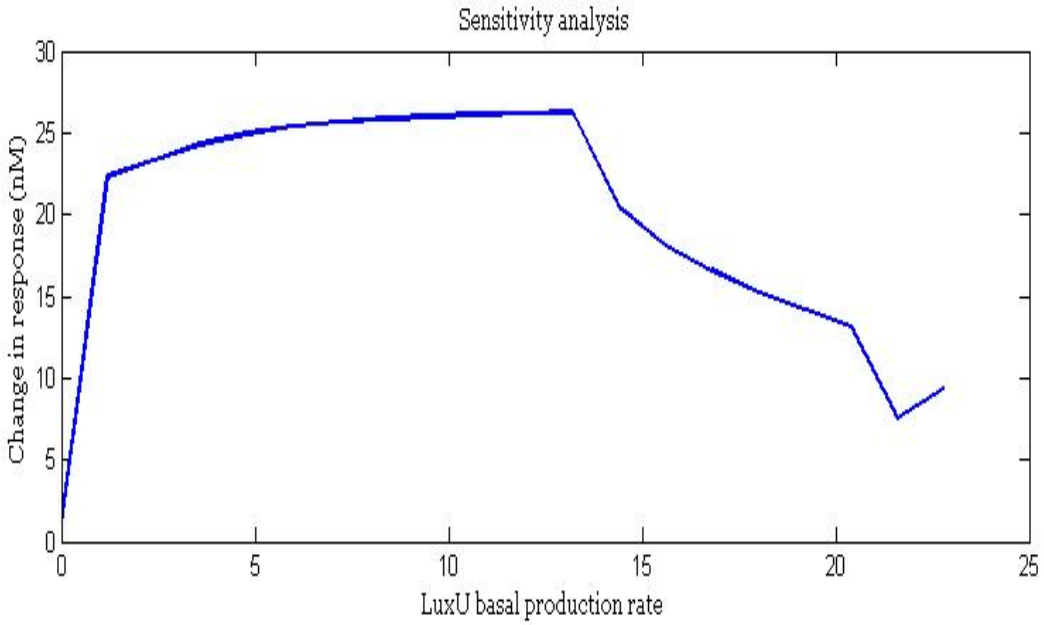

Figure 5.3 Change in time response when au varies

6. Maximum rate of TA expression

The sensitivity analysis for the maximum rate of activator expression of pTet shows that this parameter does not affect the phosphorylated LuxO significantly. On the other hand it can be seen (figure 6.1) that the higher this rate it the higher the change in the response will be which is desirable. This parameter can be easily set at the wetlab by adding a second promoter or by modifying the RBS for a stronger grip; This must be done carefully because since this can also affect several other parameters and like proved before more production doesn't always produces a wanted response.

The parameter value was set in 5 as reported in literature and it can be seen (figure 6.1) that at 5 the curve has already grown a fair amount in the y axis, which means that this parameter was chosen well.

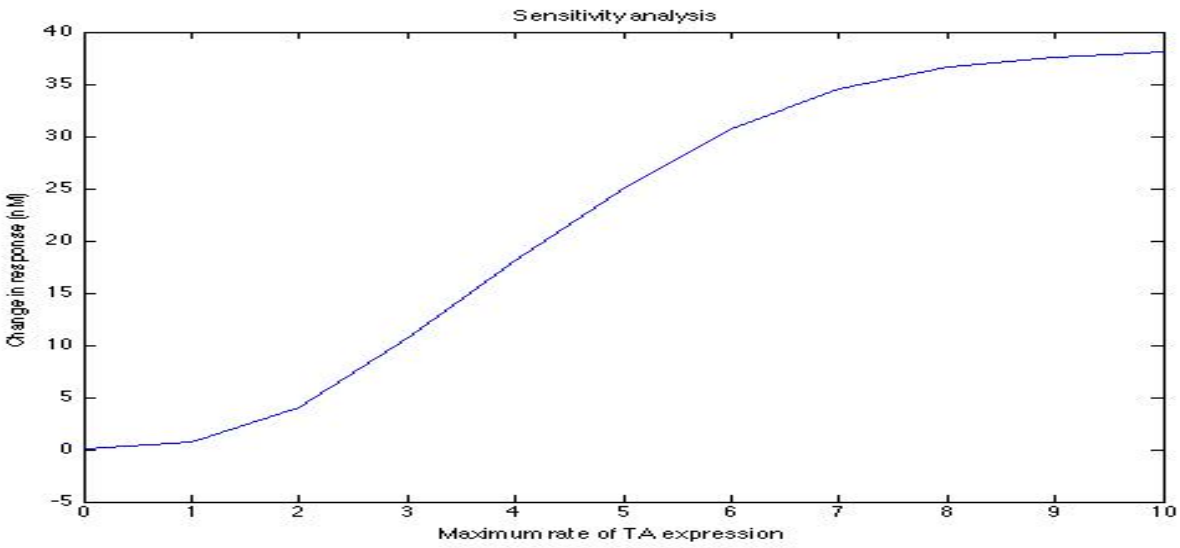

Figure 6.1 Change in response when bta varies

7. Maximum rate of TetR expression

This parameter just like the previous one is very important to obtain the desired response because it is not related to the phosphorylation cascade it has no effect in the change of phosphorylated LuxO. However it can be observed that as the maximum expression rate of the repressor raises the response gets better until the curve stabilizes at around 25 nM point at which the system probably gets saturated.

If you are paying attention at first it might seem odd that the more repressor is present in the system the better the response is, but it does make sense that the better the response molecule is being repressed the more significant the change will be.

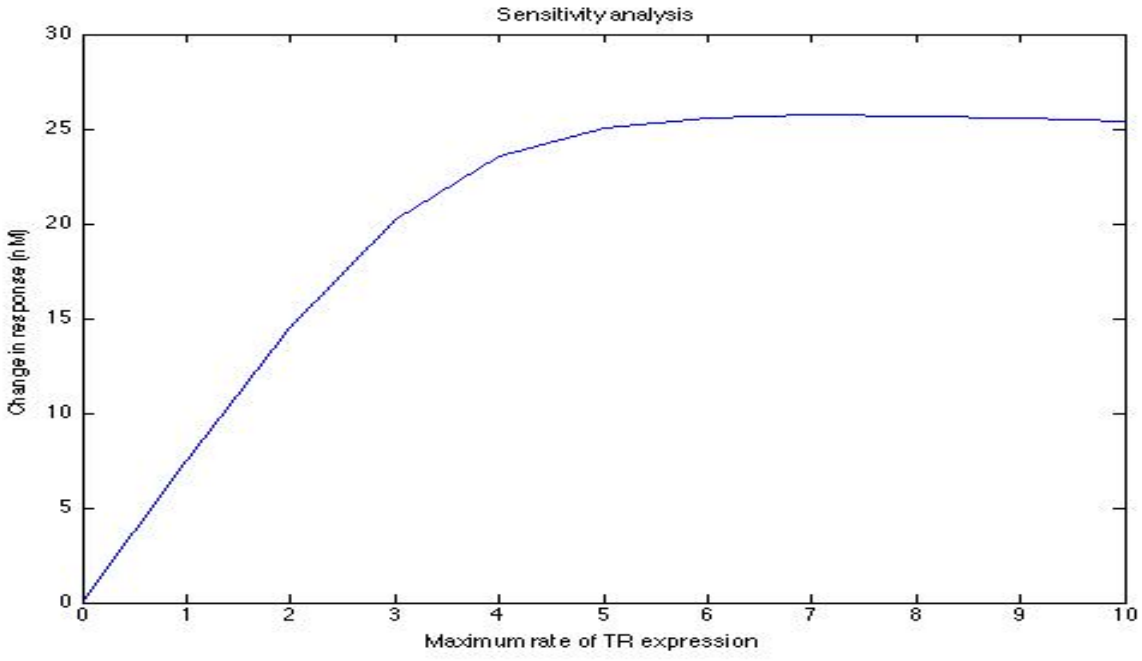

Figure 7.1 Change in response of the pTet Repressor when btr varies

8. CqsS protein decay rate

If the protein degradation rate is too high the chance that a protein will be degraded before it can reach its role in the phosphorylation cascade, and will result in a not phosphorylated version of luxO causing the system to be ON all the time. When the Autoinductor is present the change in the response will remain.

Also this parameter is pretty much fixed since it's a membrane bound protein and its decay rate is given by cell division rather than an actual process of protein degradation.

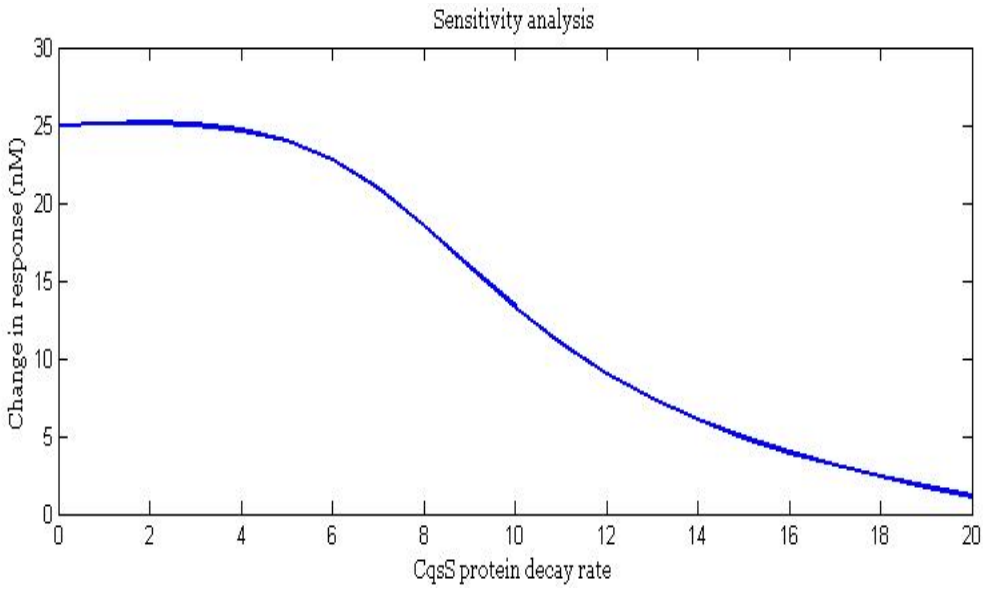

Figure 7.1 Change in response of the CqsS Repressor when gcs varies

9. CqsS* protein decay rate

In this case the activated CqsS protein decay rate has little to no effect in the response, since its values cary in less than 10% where for other parameters have been seen to be able to get as high as 20 times its original value. The fact that the response is less then the degradation rate is higher is probably because the parameter regulates the depletion of activated CqsS in the cell, the higher this parameter is the less active CqsS molecules will be available reducing the efficiency of the response.

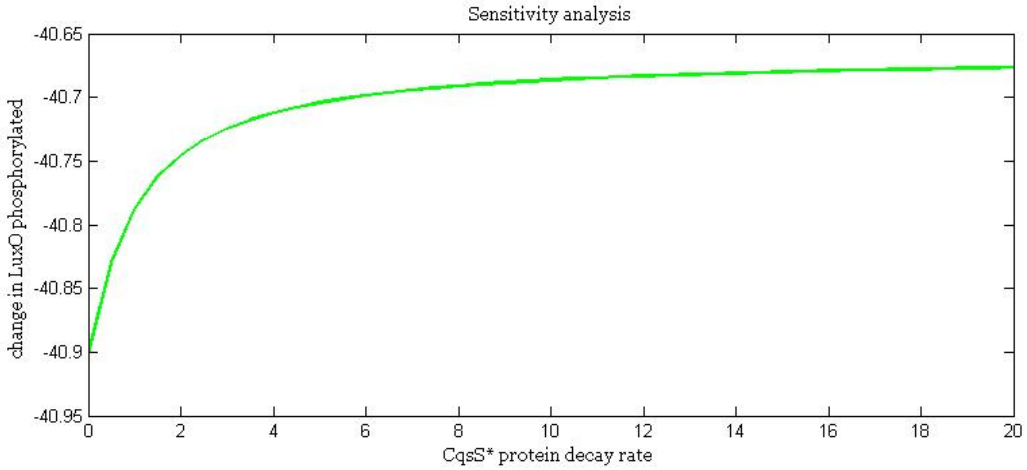

Figure 9.1 Change in phosphorylated LuxO when gcsa varies

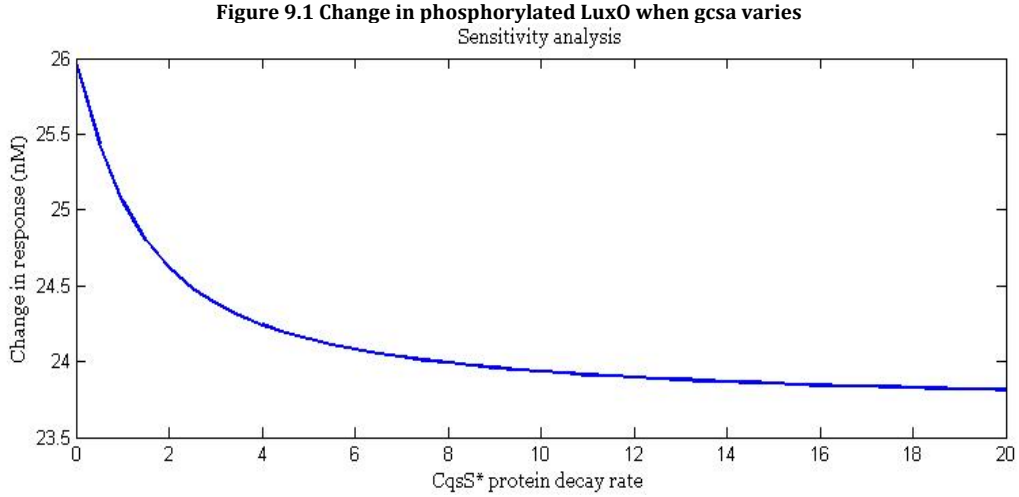

Figure 9.2 Change in response when gcsa varies

10. LuxO protein decay rate

LuxO degradation rate proves to be a significant parameter for the system; the figures below show that there is not a clear tendency for the dephosphorylation of LuxO in the system when LuxO's protein decay rate varies when assuming low values the response is stable and desirable, not the same thing happens as the values increases because the change in the response starts decreasing rapidly. The point where the change in response starts decreasing is probably the point in which the probability for the protein to be degraded before it can carry out its function is greater than the probability for the protein to carry out its function before it is degraded.

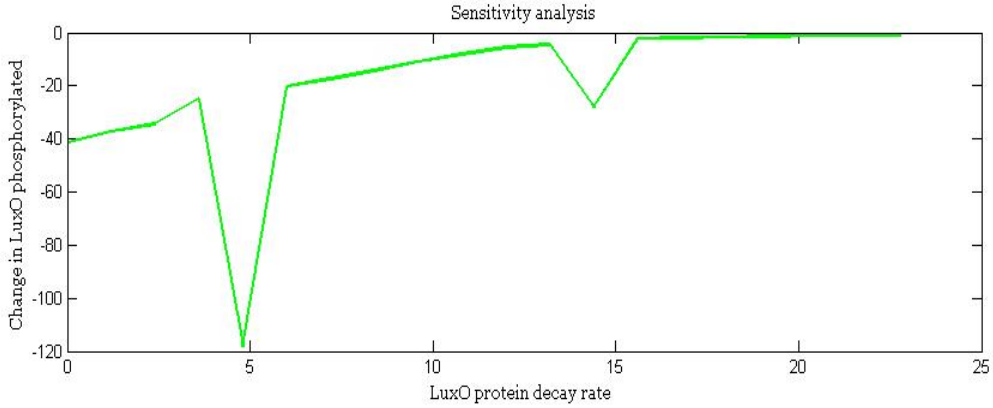

Figure 10.1 Change in phosphorylated LuxO when go varies

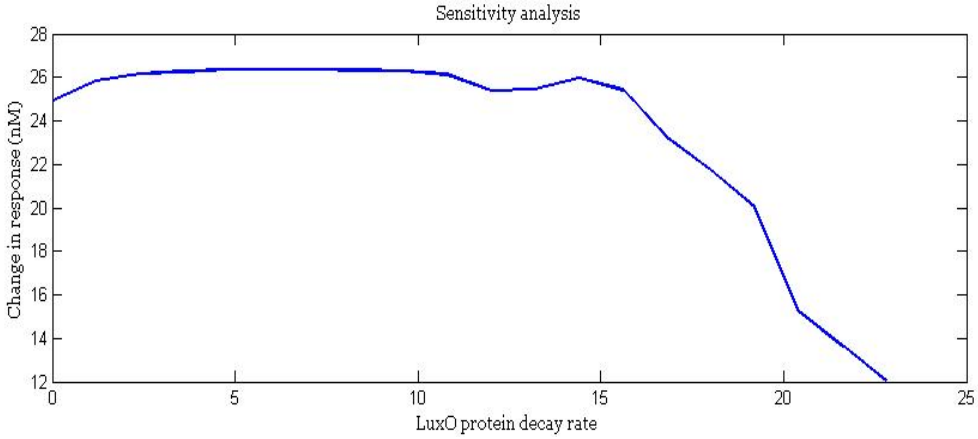

Figure 10.2 Change in response when LuxO varies

11. LuxO* Protein decay rate.

The degradation of phosphorylated LuxO is also important for the system. The maximum desired response peak is reached when the parameter assumes a value of around 1. It was expected that the higher degradation for this protein the better the response would be, since the repressor wouldn't be being produced, anyhow this can not be seen in the figures below.

The parameters of degradation of LuxO proteins proved to be of great importance for the mathematical model. In this case it is also given by cell division (0.5 h^{-1}), which is exactly where the change in response is greater (Figure 11.2)

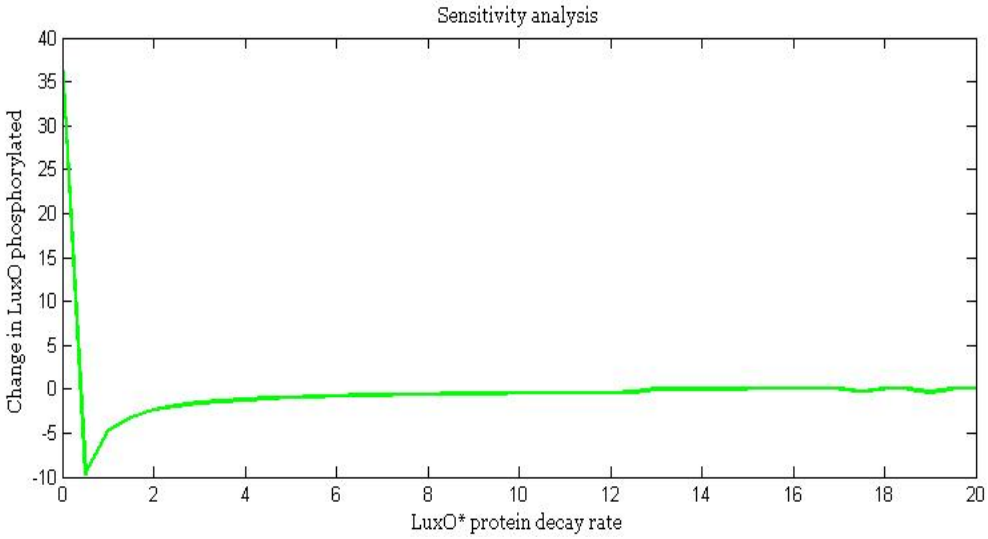

Figure 11.1 Change in phosphorylated LuxO when gof varies

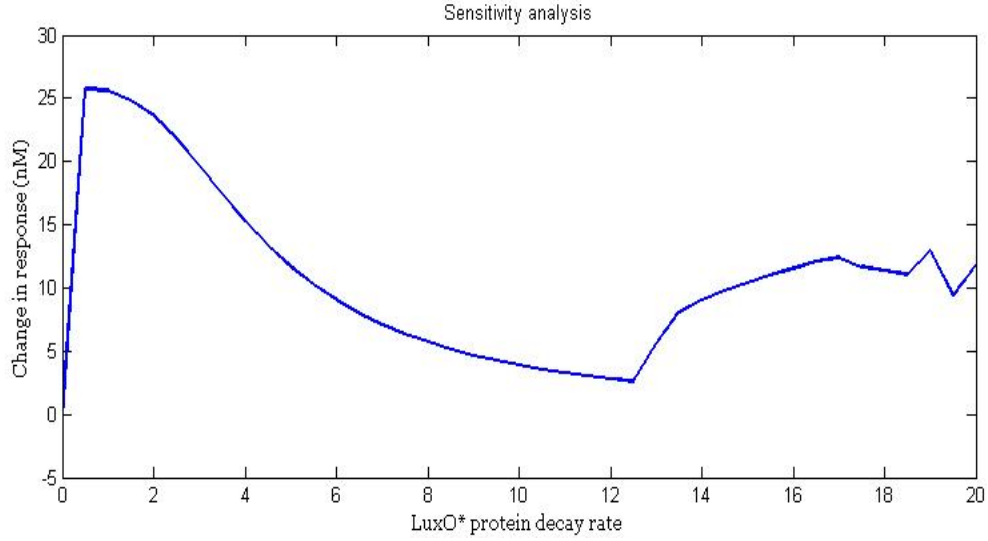

Figure 11.2 Change in response when gof varies

12. Response molecule decay rate

If the response molecule was never degraded it can be seen (figure 12.2) that the change in response would be very, very high that is if is degradation rate was 0 h^{-1} but when it assumes the value 1 h^{-1} the change in response is practically null because it would be degraded before reaching a concentration in which we would be able to detect it.

Luckily this parameter is also given by the cell division time which is 0.5h^{-1} and yields an acceptable change in the response.

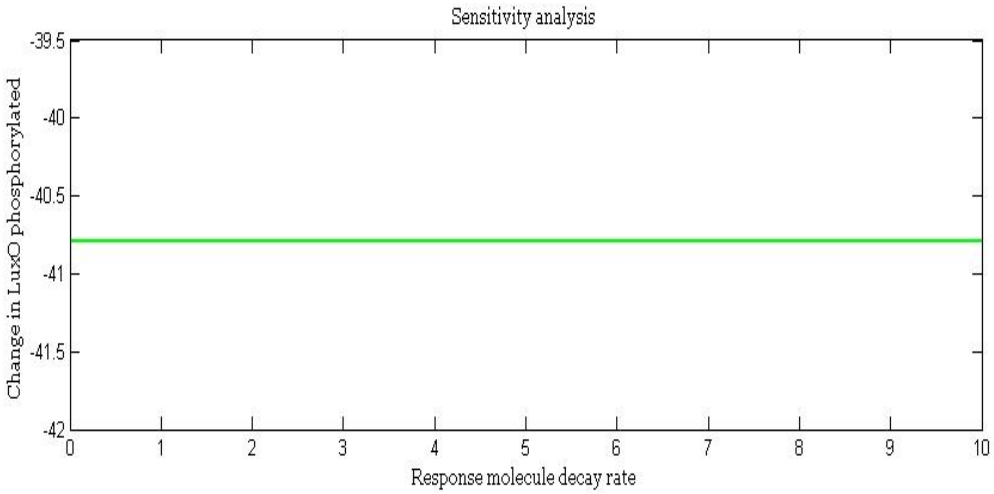

Figure 12.1 Change in phosphorylated LuxO when gr varies

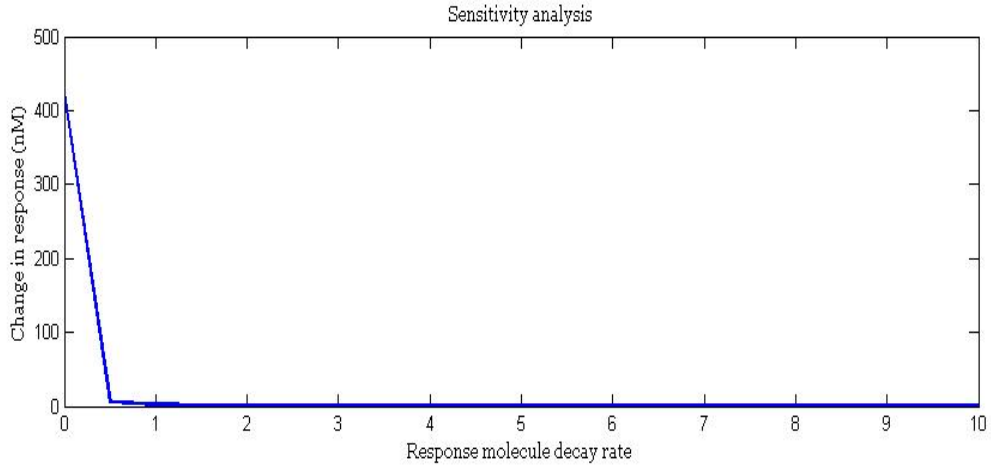

Figure12.2 Change in response when gr varies

13. PTet activator protein decay rate.

The activator decay rate dynamics are pretty much the same as the previous parameter, there is no change in phosphorylated LuxO and the form of the change in response curve is the same, although this one is not as important because the activator is not involved directly in the response, its not the molecule being detected.

Figure 13.1 Change in phosphorylated LuxO when gta varies

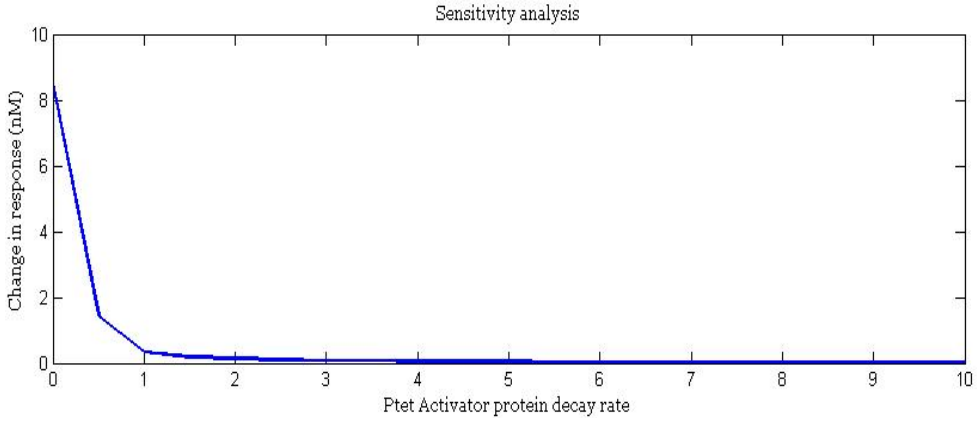

Figure 13.1 Change in response when gta varies

14. PTet repressor protein decay rate.

Again this parameter is not one of which is involved with LuxO phosphorylation, And as many other decay rates this one is given by cell division, with a peak in around 0.5 h^{-1}. The time of response graph is pretty much standard and is very high in low values. The delay this parameter brings to the system is one of those that has to be dealt with head on since it can not be changed and will ultimately define the time in which the system responds.

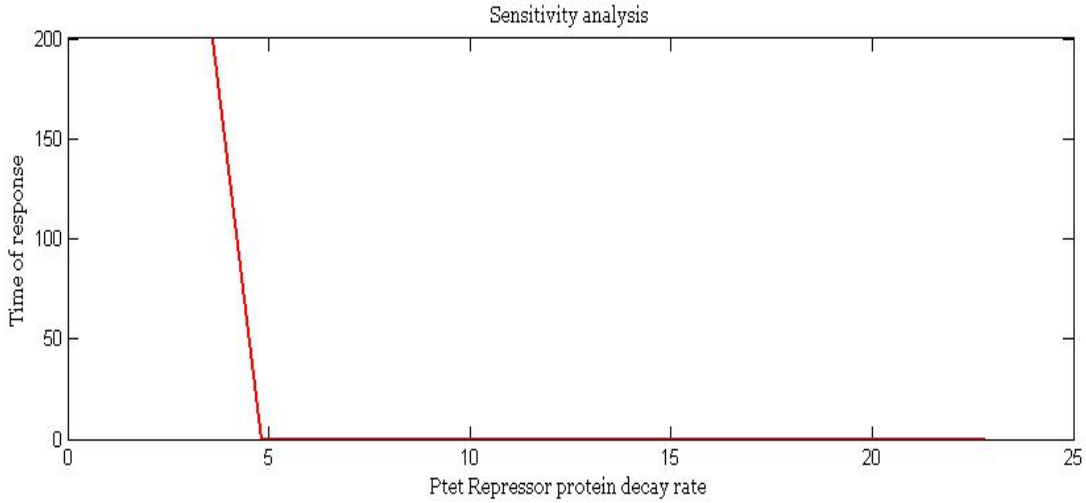

Figure 14.1 Change in response when gtr varies

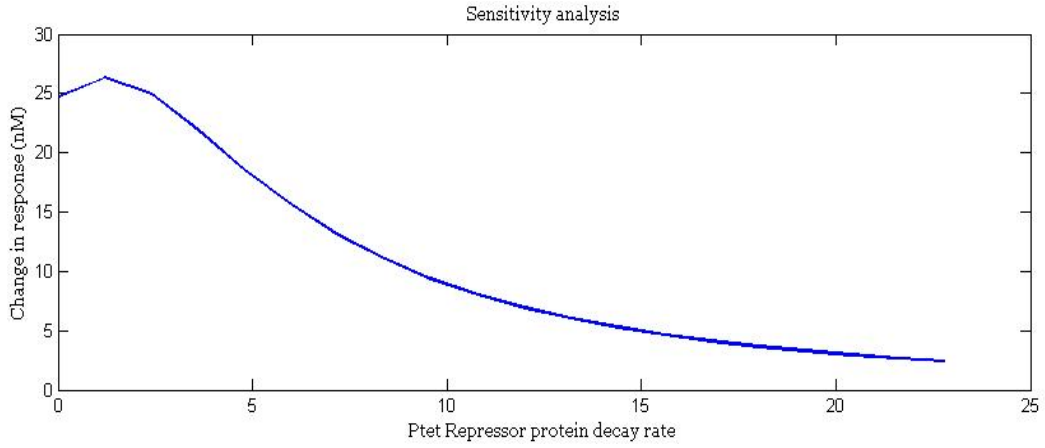

Figure 14.2 Change in response when gtr varies

15. pTet2 dimer repressor decay rate.

The dimer decay rate also is not directly involved in the time of response, as it remains a constant through different values, anyways the characteristic peak around 0.5 h^{-1} for the decay rate is clearly demarked (Figure 15.1) for this specific parameter

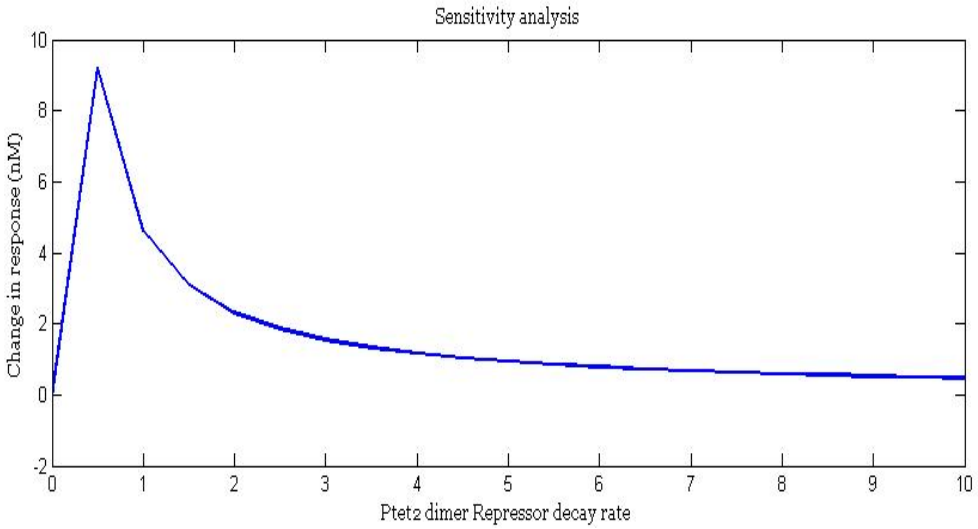

Figure 15.1 Change in response when gttr varies

16. LuxU protein decay rate

LuxU is directly involved in the phosphorylation of LuxO (for a change) by decreasing its value, so low values for the decay rate will work better to modify the amount of LuxO but only from a certain point.

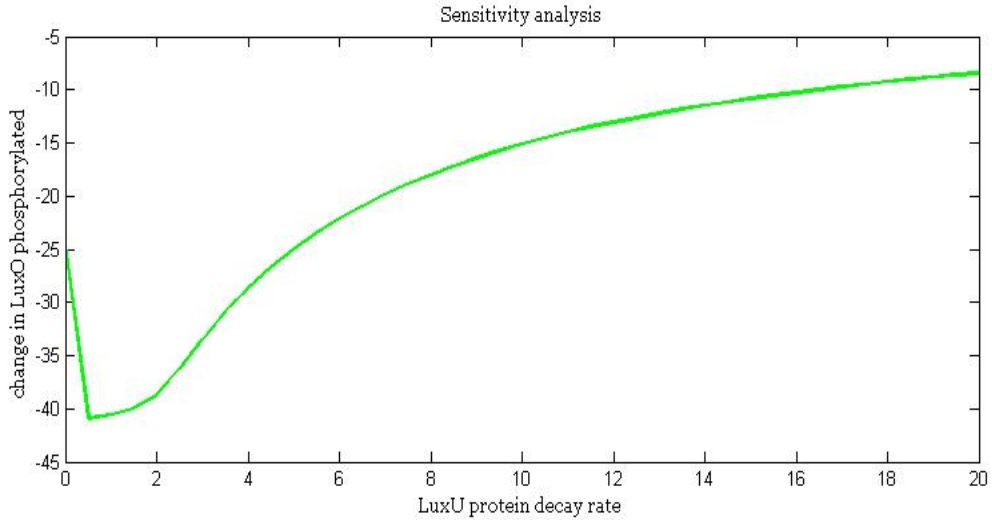

Figure 16.1 LuxO phosphorylation when gu varies

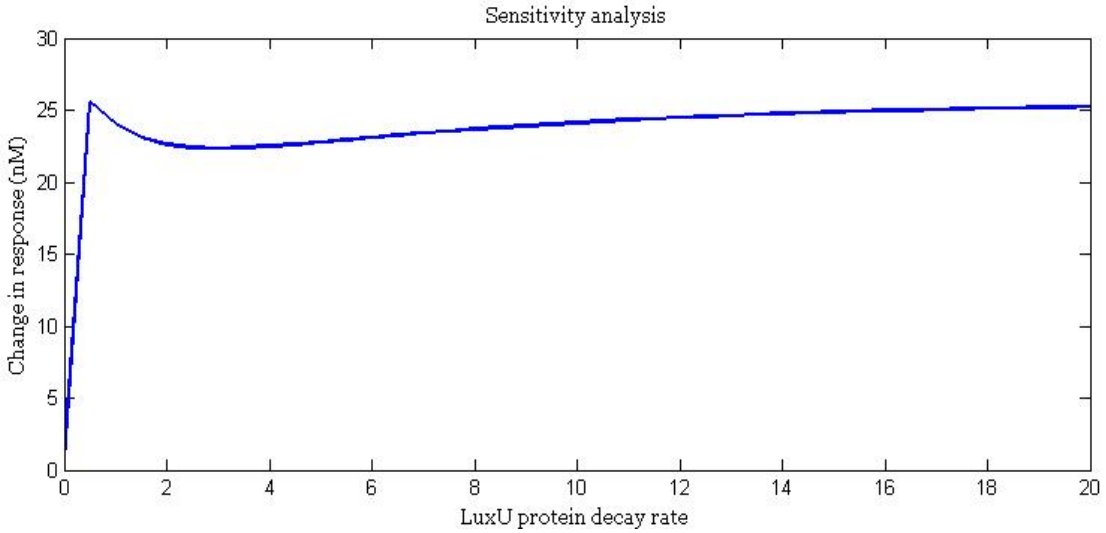

Figure 16.2 Change in response when gu varies

17. LuxU* protein decay rate

One might think that the plots for this parameter would be inverse when compared to the previous one and for the dynamics of the phosphorylated LuxO this is true, but not in the response.

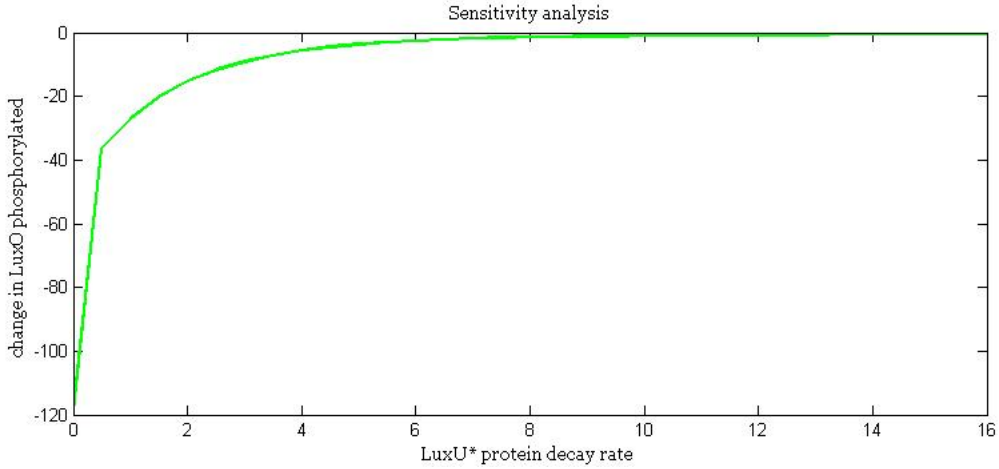

Figure 17.1 LuxO phosphorylation when guf varies

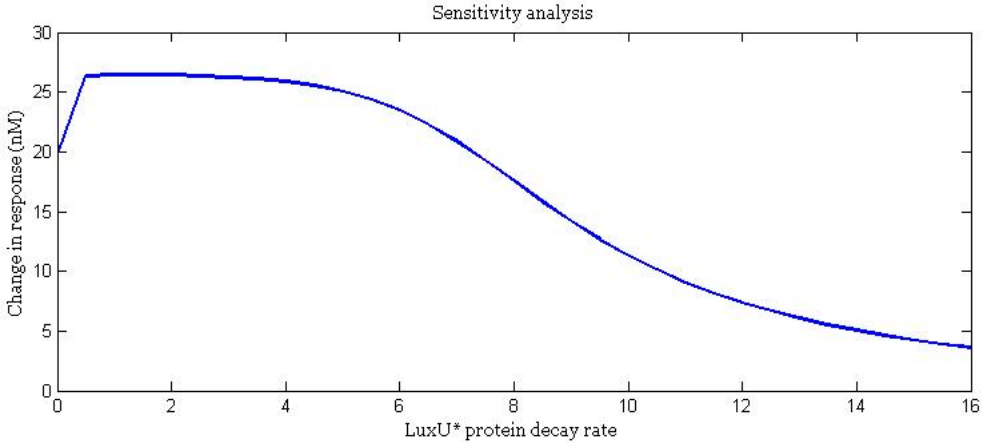

Figure 17.2 Change in response when gu varies

18. LuxO-DNA coupling rate

This is a fairly important parameter for the model although it is not involved with the phosphorylation of of LuxO (figure 18.1), it requires careful choosing because it is proportional to both the change in response and the time of response which means that if we choose a parameter value too high the change in response will be greater, but also the system would take longer to reach that point, the contrary is also true, low values for the ho parameter would yield less change in response but faster. So to correctly choose this parameter one must analyze the advantages and disadvantages and pick a point where it's just right.

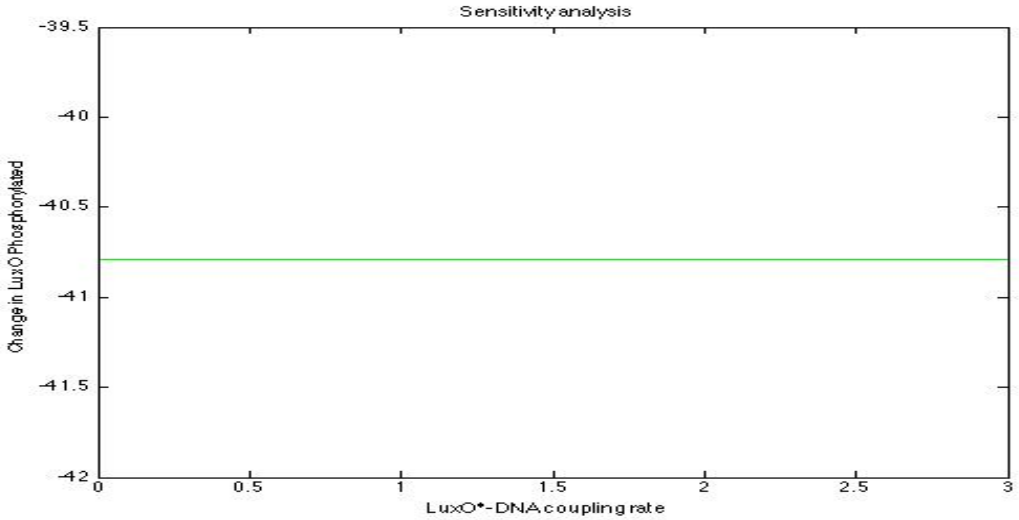

Figure 18.1 Change in phosphorylated LuxO when ho varies

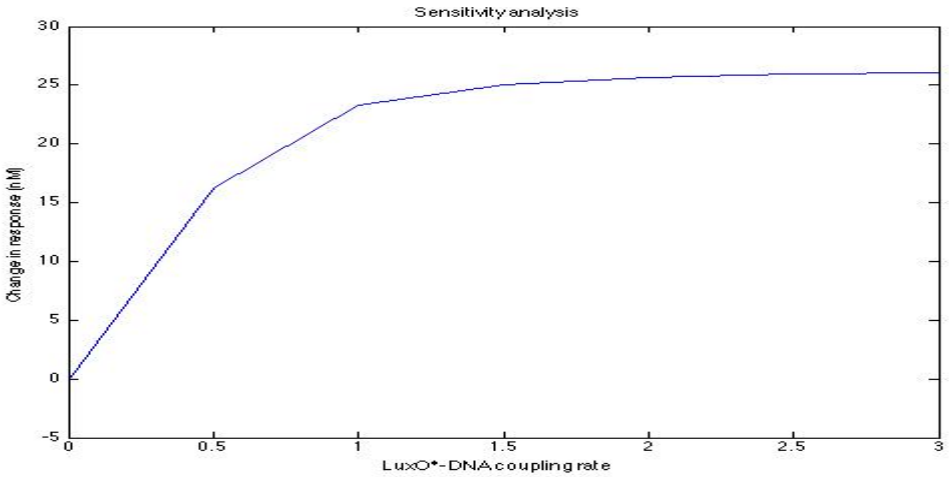

Figure 18.2 Change in response when ho varies

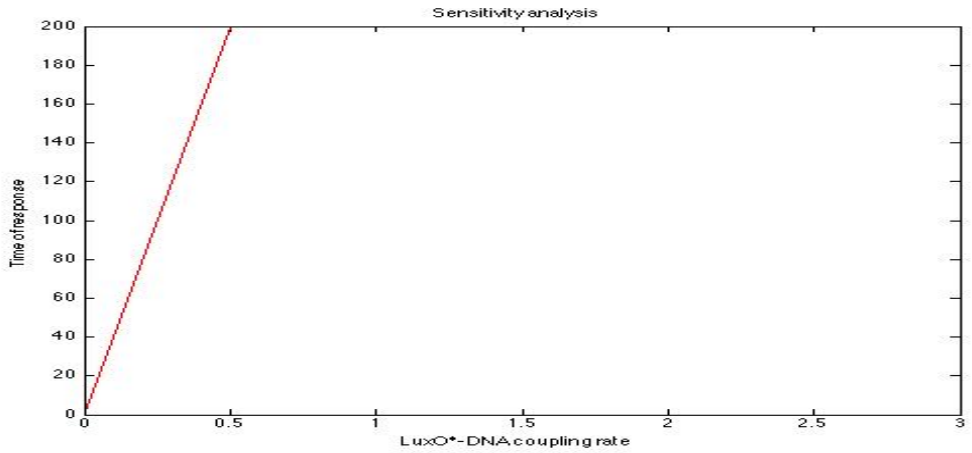

Figure 18.3 time of response when ho varies

19. TR domain-DNA coupling rate

Just like the previous parameter this one is not involved in the phosphorylation of LuxO (figure 19.1) but unlike it, the time of response remains a constant as well (figure 19.3) these type of parameter are key to maximize the change in response because it really doesn't matter what values it comes to have the only real effect they will have in the system is in the strength of the response.

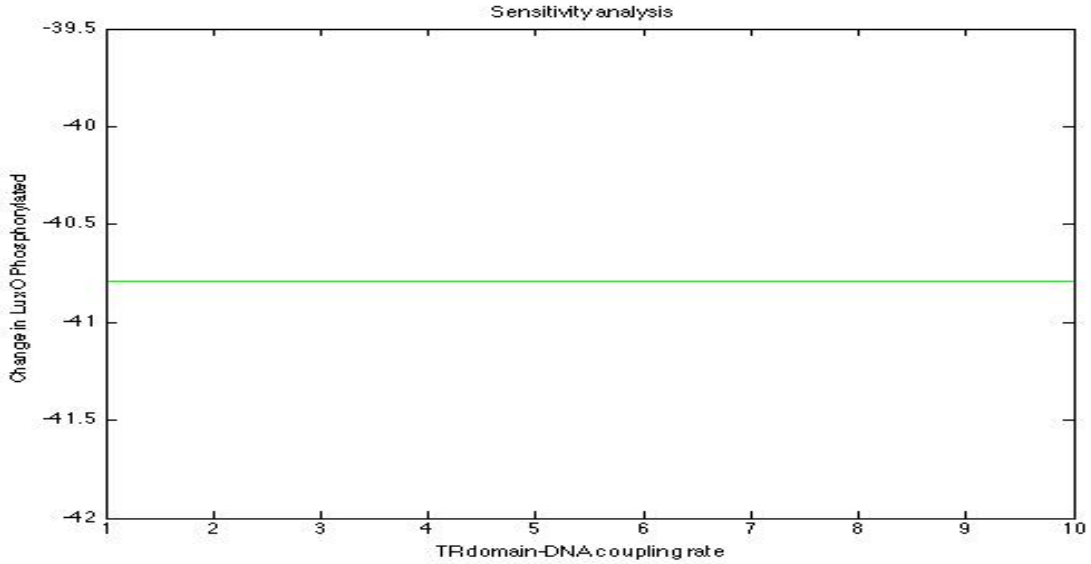

Figure 19.1 Change in phosphorylated LuxO when htr varies

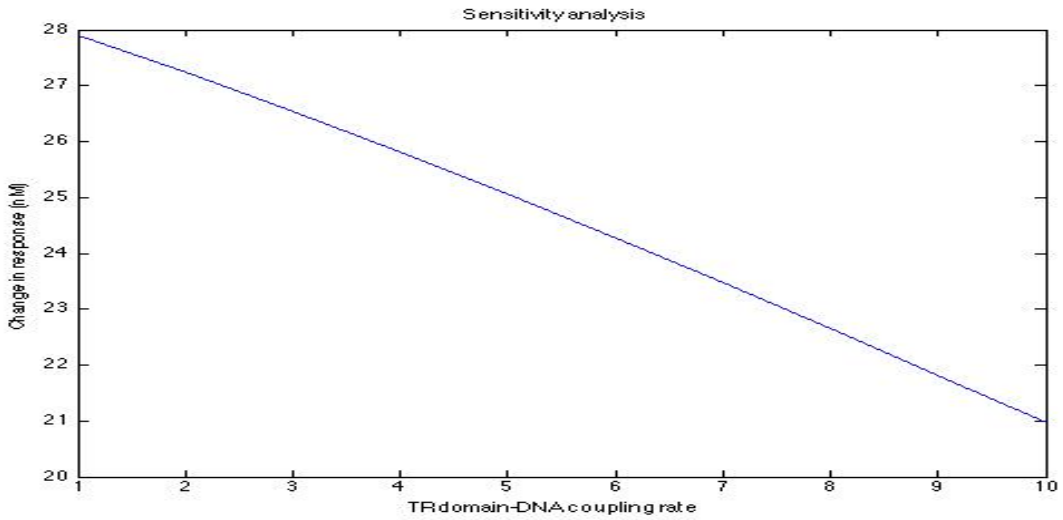

Figure 19.2 Change in when htr varies

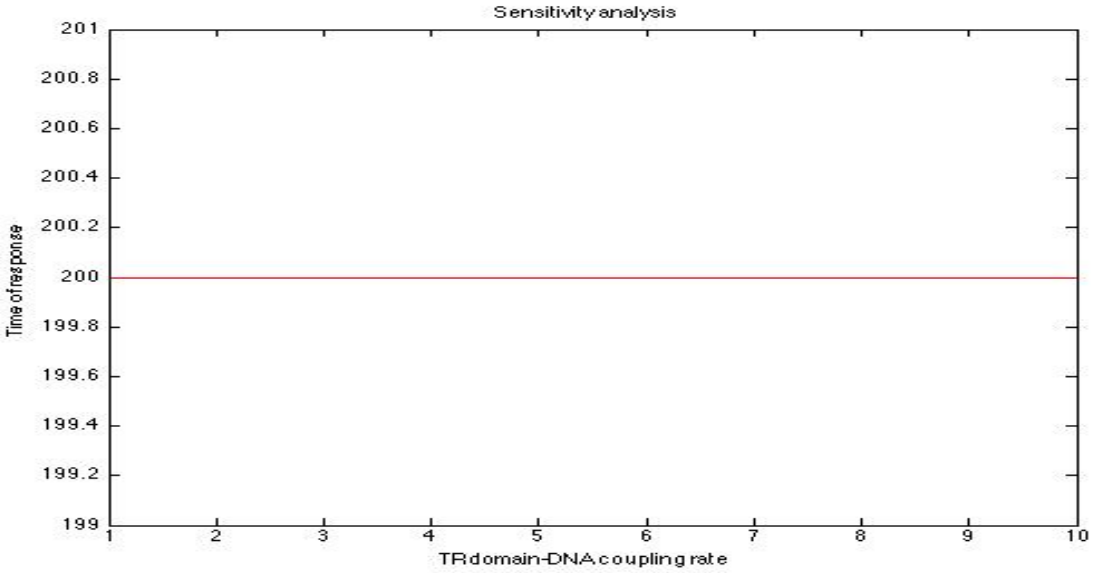

Figure 19.3 time of response when htr varies

20. CAI and CqsS decoupling rate

CqsS is the protein in charge of actually sensing the CAI, the decoupling rate gives an idea of how often the CAI isn't able bind, so the higher the decoupling rate the more concentration of CAI will be detected.

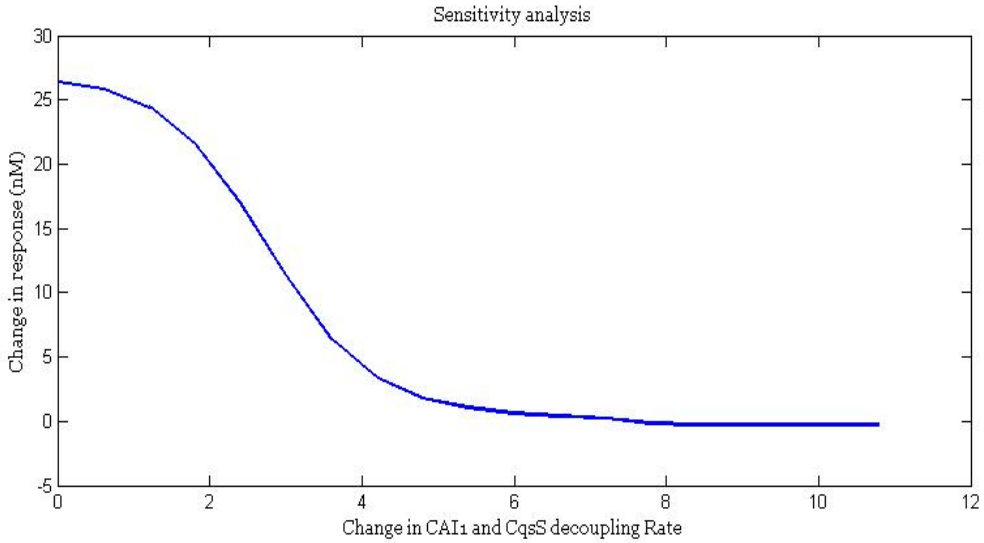

Figure 20.1 Change in response when kcd varies

21. Phosphorylation rate CqsS-LuxU

Here we can see that the change in response isn't that big (figure 21.1) by checking the numbers in the y axis, and it actually starts in a pretty high value, so pretty much any number this parameter can assume will suit our model.

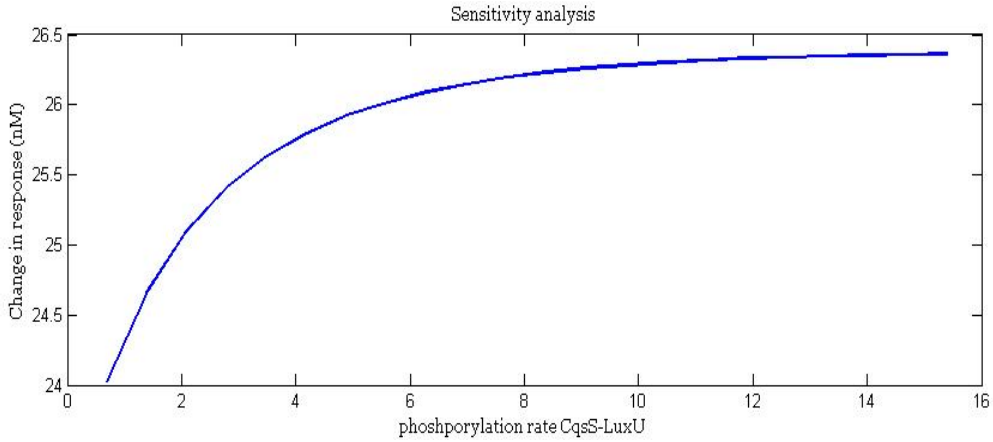

Figure 21.1 Change in when kcu varies

22. Phosphase process rate LuxU-LuxO

Results indicate that for this parameter the optimal value lies in a very small range mostly because of the response time (figure 22.2), the variation of the change in response in that specific range is practically null (figure 22.1), this tells us that the smaller the parameter is the better and faster the response will be.

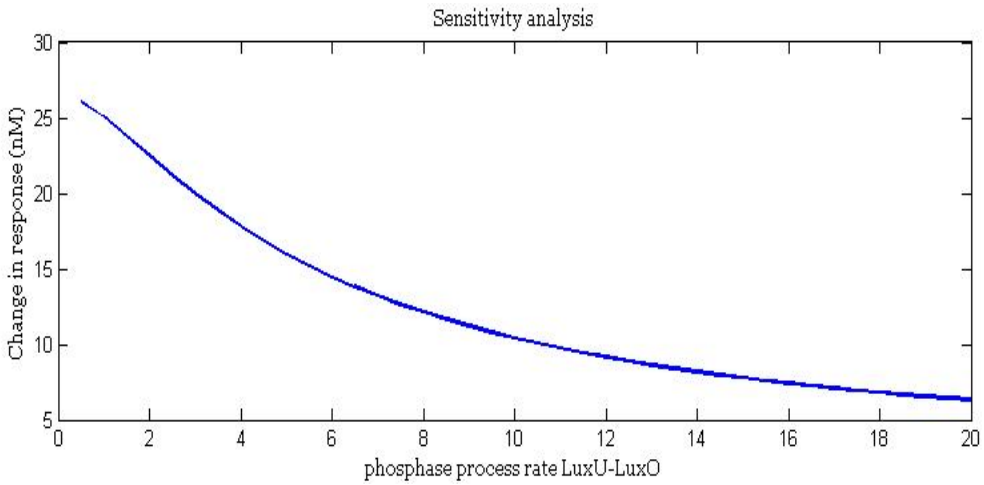

Figure 22.1 Change in when kuo varies

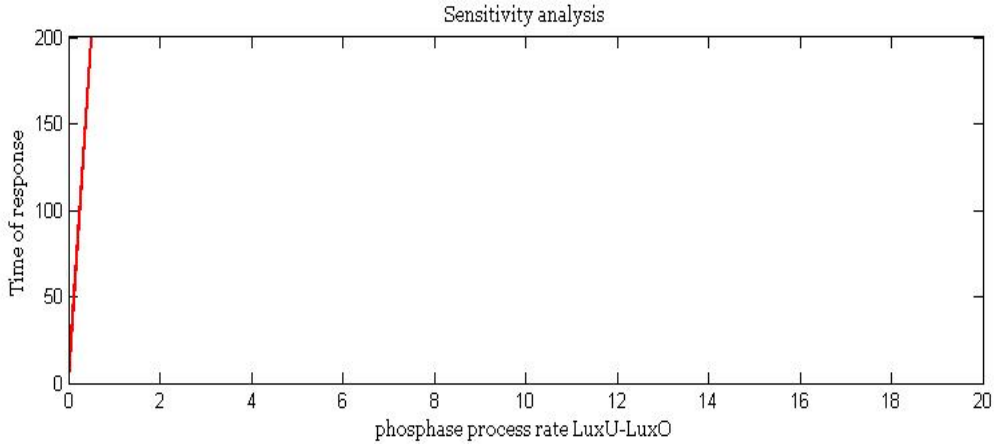

Figure 22.1 Time of response when kuo varies

23. Dimerization rate TetR

This parameter is pretty much independent of everything (figures 23.1,2) the only modification it will have in the system will be in the number of TetR dimer (which might sound trivial, but the point is if the molecule bound not in a dimer but as singles the response would be pretty much the same.

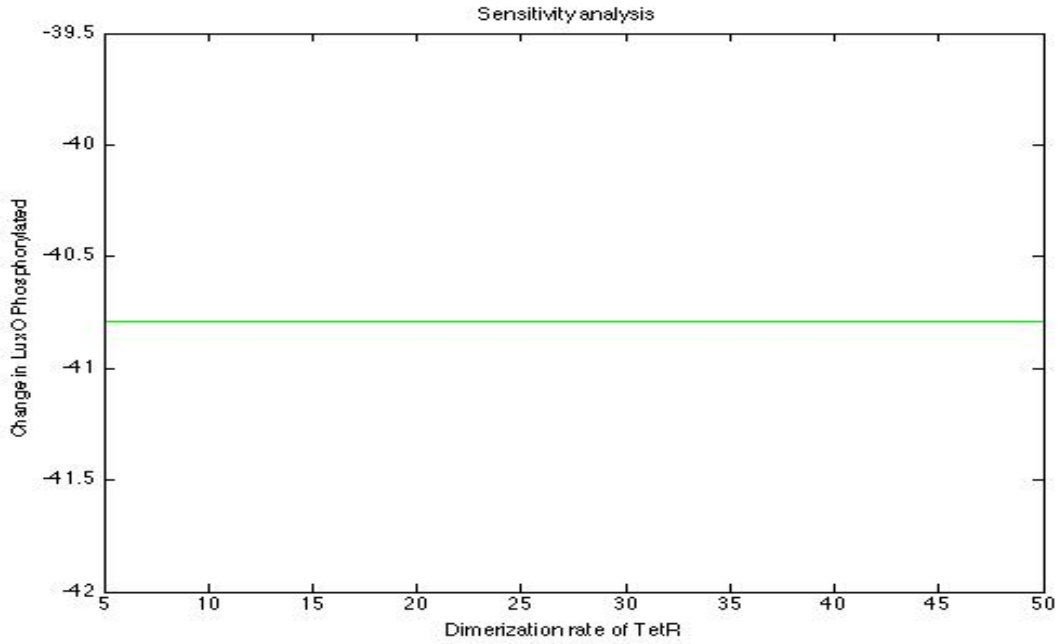

Figure 23.1 Change in phosphorylated LuxO when kttr varies

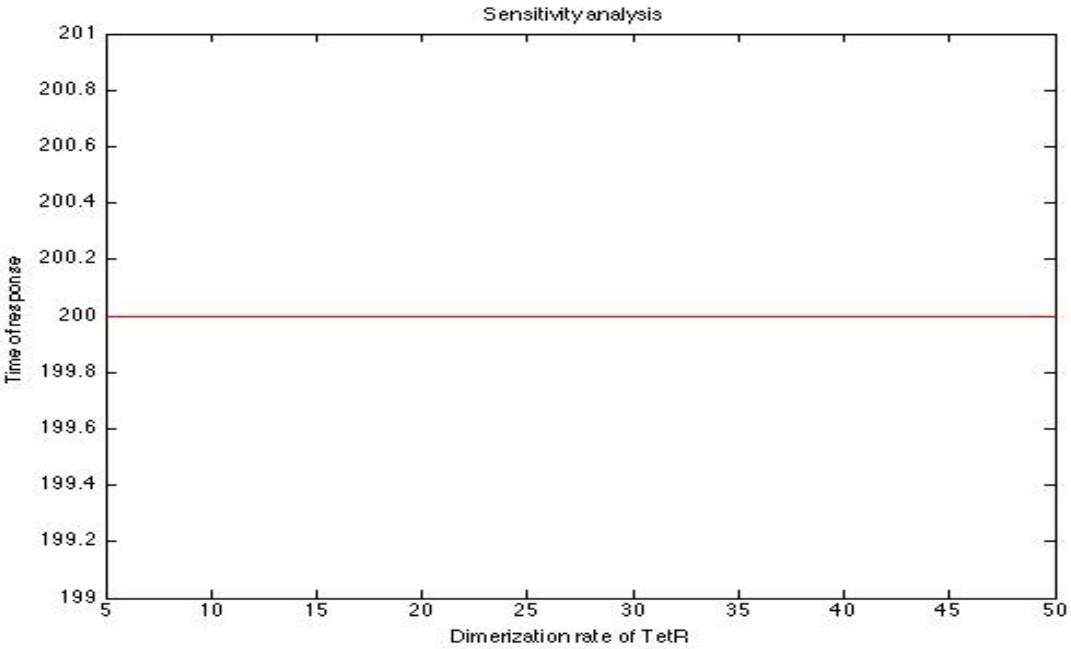

Figure 23.2 Time of response when kttr varies

24. Phosphase process rate CqsS-LuxU

Here again, the range of the parameter's acceptable value seems to be limited in a small range due to the time of response and luckily the greatest change in response is given in this range.

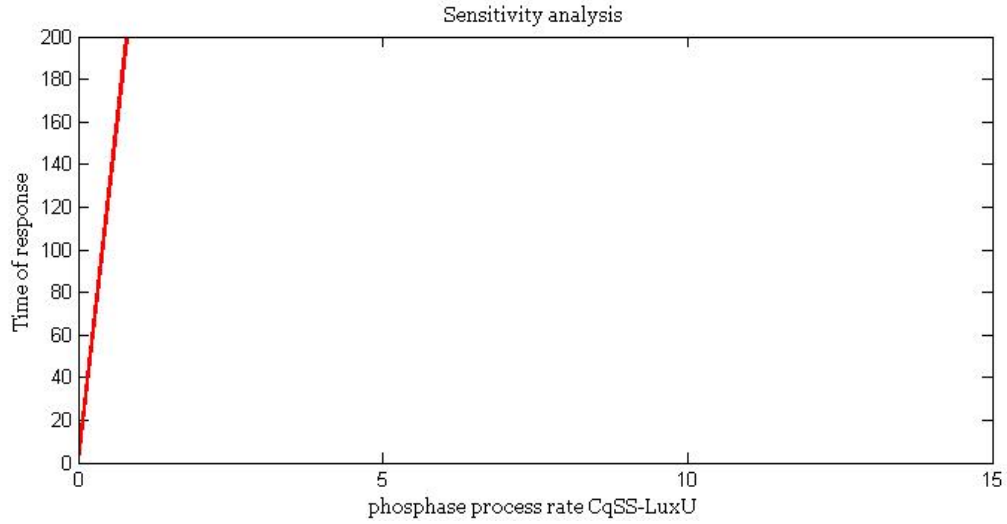

Figure 24.1 time of response when kuc varies

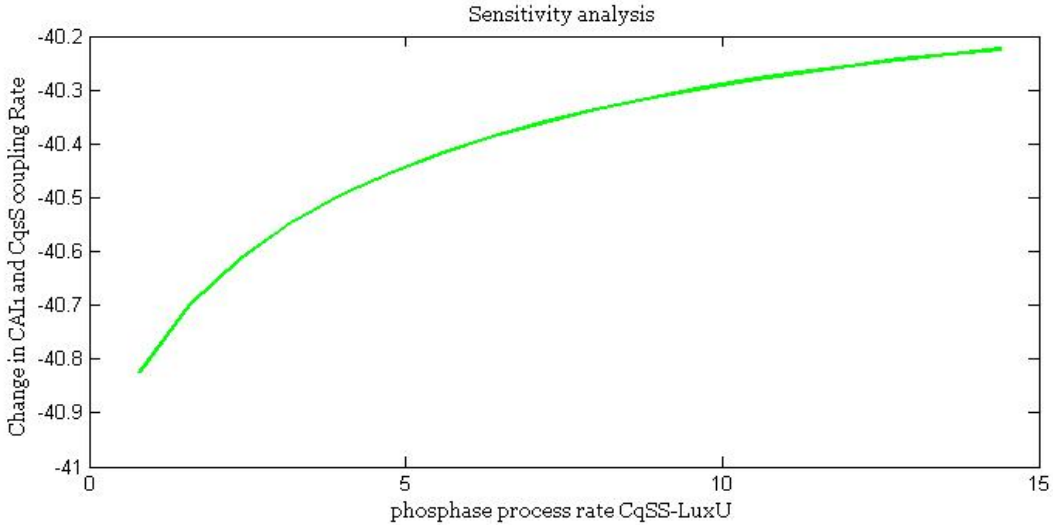

Figure 24.2 Change in phosphorylated LuxO when kuc varies

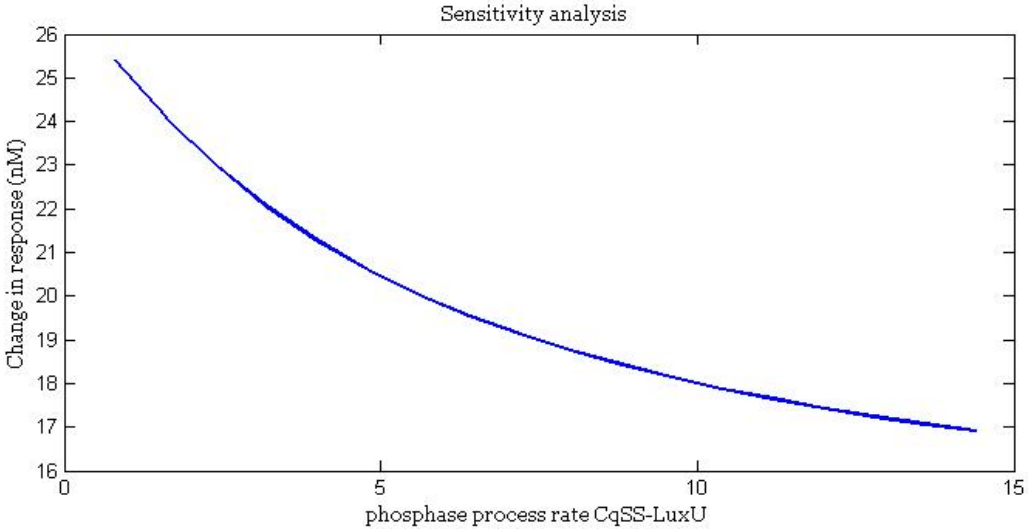

Figure 24.3 Change in response when kuc varies

25. Hill's coefficient

The hill coefficient is also one of the other parameters available to modify in the wetlab it can usually assume values between 1 and 4, throughout these values the phosphorylated LuxU remains constant as does the time of response of the system, and although it does increase the change in the response with increasing values for n the increment isn't that much so pretty much any value between 2 and 4 will suit the model well.

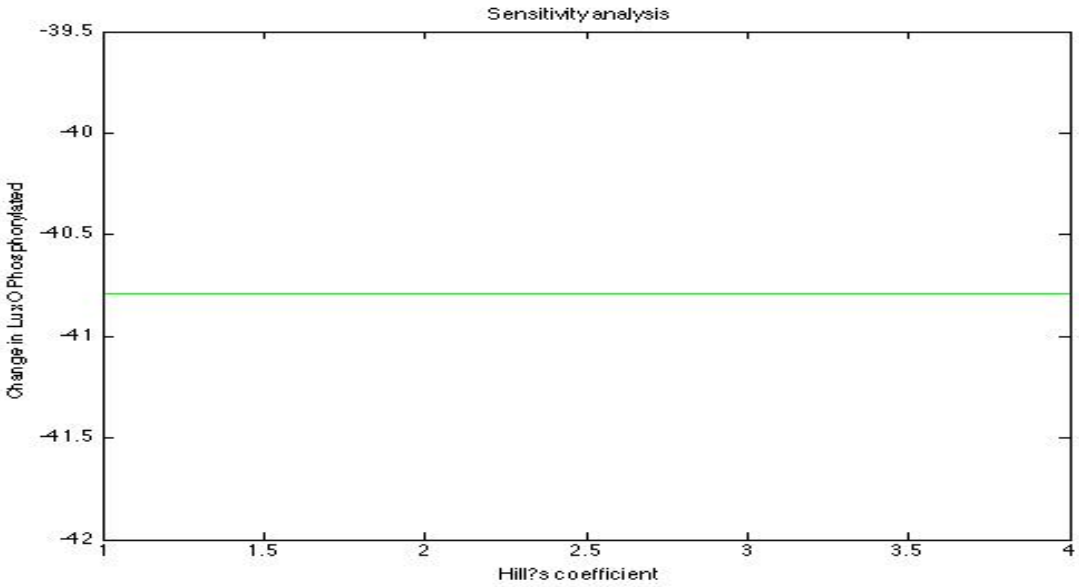

Figure 23.1 Change in phosphorylated LuxO when n varies

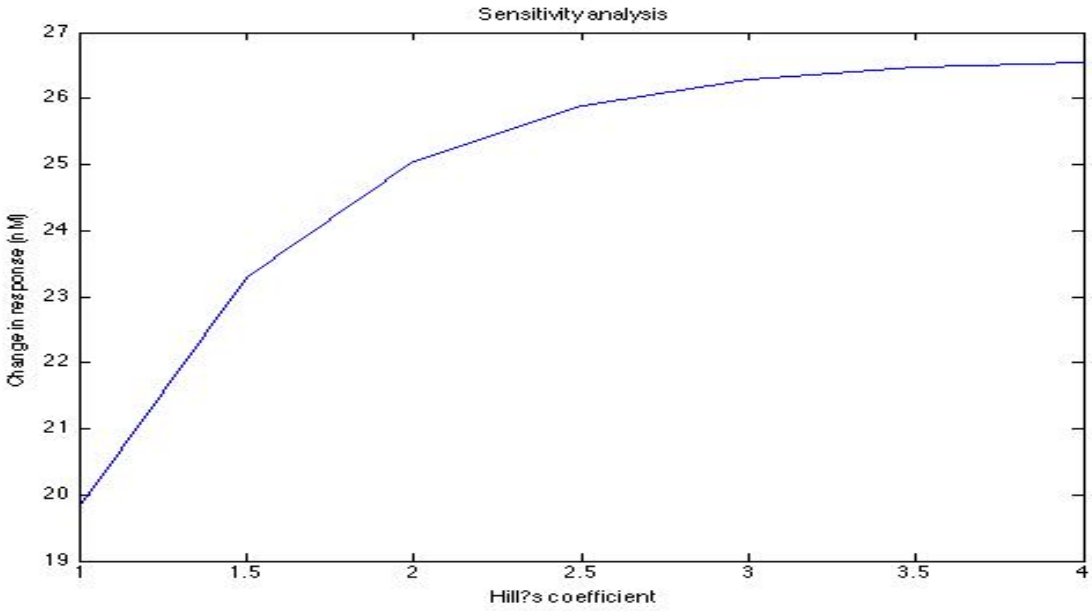

Figure 23.1 Change in response when n varies

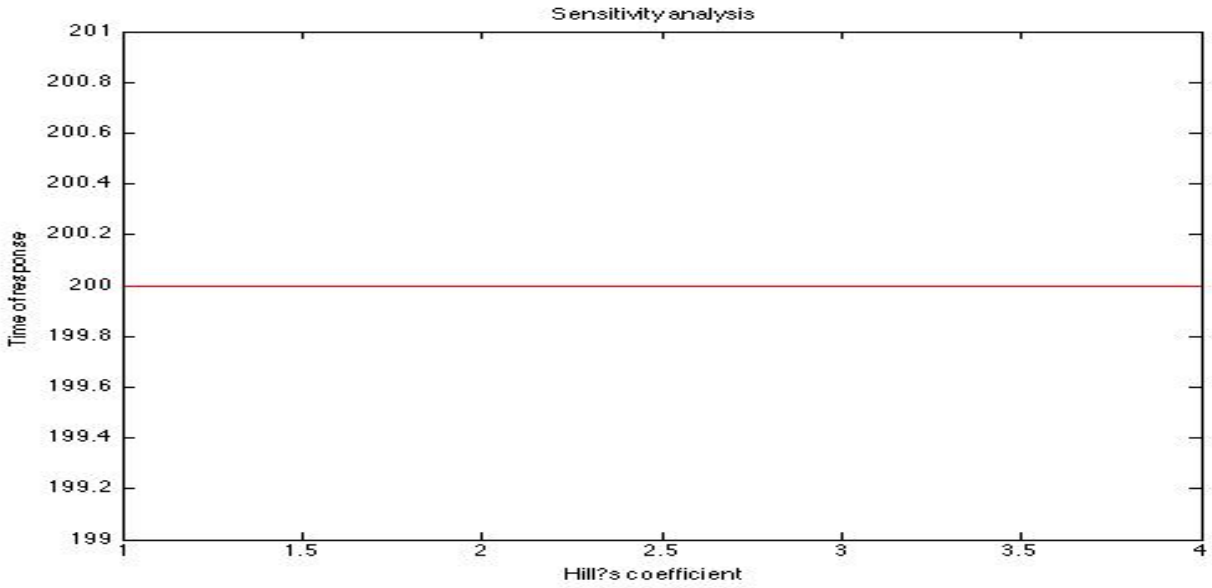

Figure 23.1 Time of response when n varies