ISSN (Online) : 2319 - 8753
ISSN (Print) : 2347 - 6710

International Journal of Innovative Research in Science, Engineering and Technology

Volume 3, Special Issue 3, March 2014

2014 International Conference on Innovations in Engineering and Technology (ICIET'14)
On 21st & 22nd March Organized by

K.L.N. College of Engineering, Madurai, Tamil Nadu, India

Analytical Solutions of Fluroscent Reporters to auxin influx in plantcells

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ABSTRACT— A Mathematical model of biosensors for phytohormone quantification has been developed. The model is based on the system of five coupled non-linear ordinary differential equations that describes the dynamics of DII-VENUS transcriptional reporter degradation in response to external application of Auxin . The nonlinear relationship that exists between the target molecule and the reporter necessitates the development of mathematical models which are used to relate the sensor fluorescence with hormone abundance quantitatively. In this article the analytical expressions for various levels of degradation of DII-VENUS for various doses of Auxin have been derived by using homotopy perturbation method. The analytical results are compared with simulation results using Mat lab program. It is found that the numerical results agree with the analytical results and with the appropriate theories.

KEYWORDS— Auxin , Transcriptional reporters, DII-VENUS, Phytohormone Quantification, Homotopy Pertubation method.

I. INTRODUCTION

In recent years there are impressive advances in technologies that are available to detect signalling molecules in living cells. Plant hormones are signalling molecules which coordinate all the aspects of plant growth and development. The biologists have been attracted towards the study of molecular mechanism for signal transduction of plant hormones during the last two decades. Remarkable progress has been made in identifying transcriptional receptors and key signaling components of plant hormones[1] . Auxin is a unique plant hormone which moves actively around the plant by a series of transmembrane pumps or by the pump components [2]. The dynamic distribution of the auxin hormone within the plant tissues controls an variety of developmental processes.[3]. The growth of the root is regulated by targeting Aux/IAA repressor proteins for degradation by auxin. In this part of the work the conjunction of the mathematical model with Aux/IAA based reporter DII-VENUS, is used to quantify Auxin abundance in plant hormones. Auxin acts by promoting the interaction between its receptors TIR1/AFB1-3 and Aux/IAA repressor proteins [4–6], resulting in their ubiquitination and degradation [7–9]. Having captured the quantitative and temporal relationship between DII-VENUS and Auxin levels in the network model, researchers will now be able to use live imaging to follow the dynamics of DII-VENUS distribution in plant tissues and extrapolate them to changes in Auxin levels. Bandet al. [10] demonstrated that this was possible by studying dynamic changes of DII-VENUS reporter.

II. MATHEMATICAL FORMULATION OF THE PROBLEM

Auxin promotes the degradation of DII-VENUS by mediating its ubiquitination. In each cell this degradation occurs via the network of interactions as shown below.

[Auxin] + [TIRI] ↔ [Auxin.TIRI]

Here the process is modeled by assuming that the auxin-TIRI/AFB complex binds to DII-VENUS to form an auxin-TIRI/AFB-VENUS complex, which then dissociates into an ubiquiting-tagged DII-VENUS and auxin-TIRI/AFB complex.

[Auxin.TIRI] + [VENUS] ↔ [Auxin.TIRI.VENUS]

The dynamics of DII-VENUS degradation in response to external application of Auxin can be described by a system of five coupled non linear ordinary differential equation as shown below.

$$\frac{d[Auxin]}{dt} = k_d[Auxin.TIRI] - k_a[Auxin][TIRI] + \alpha - \mu[Auxin]$$
$$\frac{d[TIRI]}{dt} = -k_a[Auxin][TIRI] + k_d[Auxin.TIRI]$$
$$\frac{d[Auxin.TIRI]}{dt} = k_d[Auxin][TIRI] - k_d[Auxin.TIRI] + (l_d + l_m)[Auxin.TIRLVENUS] - l_a[Auxin.TIRI][VENUS]$$
$$\frac{d[Auxin.TIRLVENUS]}{dt} = l_a[Auxin.TIRI][VENUS] - (l_a + l_m)[Auxin.TIRLVENUS]$$
$$\frac{d[VENUS]}{dt} = \delta - l_a[VENUS][Auxin.TIRI] + l_d[Auxin.TIRLVENUS] - \lambda[VENUS]$$

The simplest form of the above set of equations for easy solving is as below follows.

$$\frac{dX_1}{dt} = k_d X_3 - k_a X_1 X_2 + \alpha - \mu X_1$$
$$\frac{dX_2}{dt} = -k_a X_1 X_2 + k_d X_3$$
$$\frac{dX_3}{dt} = k_a X_1 X_2 - k_d X_3 + (l_a + l_m) X_4 - l_a X_3 X_5$$
$$\frac{dX_4}{dt} = l_a X_3 X_5 - (l_d + l_m) X_4$$
$$\frac{dX_5}{dt} = \delta - l_a X_5 X_3 + l_d X_4 - \lambda X_5$$

$x_1 =$ [Auxin] $x_2 =$ TIRI $x_3 =$ [Auxin.TIRI],
$x_4 =$ [Auxin.TIRI.VENUS] $x_5 =$ [VENUS] (1)

When t=0, $x_1 = 1$, $x_2 = 1, x_3 = 1, x_4 = 1$ & $x_5 = 1$

The analytical expressions which are obtained from the above set of equations (2) by using homotopy perturbation method are given below

$$X_1(t) = \left(1 - \frac{\alpha}{\mu}\right) e^{-\mu t} + \left(\frac{\alpha}{\mu}\right) + \left(\frac{K_a \alpha}{\mu^2} - \frac{K_d}{\mu - K_d}\right) e^{-\mu t} - \left(\frac{K_a \alpha}{\mu^2}\right) + \left(\frac{K_d e^{-K_d t}}{\mu - K_d}\right) - \left[K_a t\, e^{-\mu t}\left(1 - \frac{\alpha}{\mu}\right)\right]$$

$$X_2(t) = 1 + \left(\frac{K_a e^{-\mu t}}{\mu}\right)\left(1 - \frac{\alpha}{\mu}\right) - \left(\frac{K_a \alpha t}{\mu}\right) - e^{-K_d t} + 1 - \left(\frac{K_a}{\mu}\right)\left(1 - \frac{\alpha}{\mu}\right)$$

$$X_3(t) = e^{-K_d t} + C e^{-K_d t} + \left(\frac{\alpha K_a}{\mu}\right) + \left[\left(\frac{K_a e^{-\mu t}}{(K_d - \mu)}\right)\left(1 - \frac{\alpha}{\mu}\right)\right] - \left(\frac{I_a t e^{-\mu t}}{\lambda}\right) + \left(\frac{I_a e^{-(K_d + \lambda) t}}{\lambda}\left(1 - \frac{\delta}{\lambda}\right)\right) + \left[\frac{(I_d + I_m) e^{-(I_d + I_m) t}}{(K_d - I_d - I_m}\right]$$

$$X_4(t) = e^{-(I_d + I_m) t} - \left[\frac{I_a\left(1 - \frac{\delta}{\lambda}\right)}{(I_d + I_m - K_d - \lambda)}\right] e^{-(I_d + I_m) t} - \left(\frac{I_a \lambda}{\lambda\,(I_d + I_m - K_d}\right) e^{-(I_d + I_m) t} + \left[\frac{I_a\left(1 - \frac{\delta}{\lambda}\right) e^{-(K_d + \lambda) t}}{(I_d + I_m - K_d - \lambda)}\right] + \left(\frac{I_a \lambda\, e^{-K_d t}}{\lambda\,(I_d + I_m - K_d}\right)$$

$$X_5(t) = \left(1 - \frac{\delta}{\lambda}\right) e^{-\lambda t} + \left(\frac{\delta}{\lambda}\right) + B\, e^{-\lambda t} + \left(\frac{I_d\, e^{-(I_d + I_m) t}}{\lambda - I_d - I_m}\right) + \left[\frac{I_a\, e^{-(K_d + \lambda) t}\left(1 - \frac{\delta}{\lambda}\right)}{K_d}\right] - \left(\frac{I_a \delta\, e^{-K_d t}}{\lambda(\lambda - K_d)}\right)$$

Where the constants

$$C = -\left\{\left(\frac{\alpha K_a}{\mu}\right) + \left[\left(\frac{K_a}{(K_d - \mu)}\right)\left(1 - \frac{\alpha}{\mu}\right)\right] + \left(\frac{I_a}{\lambda}\left(1 - \frac{\delta}{\lambda}\right)\right) + \left[\frac{(I_d + I_m)}{(K_d - I_d - I_m}\right]\right\}$$

$$B = -\left(\frac{I_d}{\lambda - I_d - I_m}\right) + \left[\frac{I_a\left(1 - \frac{\delta}{\lambda}\right)}{K_d}\right] - \left(\frac{I_a \delta}{\lambda(\lambda - K_d)}\right) \quad (2)$$

III. RESULTS AND DISCUSSION

Mathematical models are essential for reporters to become truly quantitative. The relationship between the concentration of a signaling molecule and a reporter is generally nonlinear. In figure 1 the flurosecence response of DII-VENUS reporter in different time intervals are plotted. From figure 1 it can be inferred that there is a gradual reduction in the level of DII-VENUS reporter fluroscence and it reaches its steady state after 120 minutes.

Fig.2(a) reveals that the kinectic response of DII-VENUS reporter increases gradually with the increase in the dose of auxin added and reaches its steady state. Fig.2(b) gives the relationship between the auxin dose and the corresponding auxin influx rates. It is noted that as expected, the influx rate increases with larger doses and appears to increase linearly with the doses. The relationship saturates at higher doses.

IV. CONCLUSION

Here it is reported that how the auxin reporter DII-VENUS can be used to quantify auxin abundance during a rapid developmental response .The mathematical modelling discussed here is used to understand the dynamics of the auxin response due to the degradation of

DII-VENUS reporter and also to compare the similarity of responses between different plant tissues and it is also driving the development of new biosensors and paving the way for a quantitative analysis of biological processes. Such biosensors will be helpful to understand the operation and interaction of the plants with their environment. In future this quantitative approach can be adapted for use with equivalent reporters which operate in other plant hormone response pathways to develop further mechanistic insights.

The Basic concept of Homotopy perturbation method which is applied for finding the analytical solutions of the differential equations involved is given in Appendix A and the Mat lab program used for finding the numerical solutions of the equations is given in Appendix B.

V. FIGURES AND TABLES

Fig.1

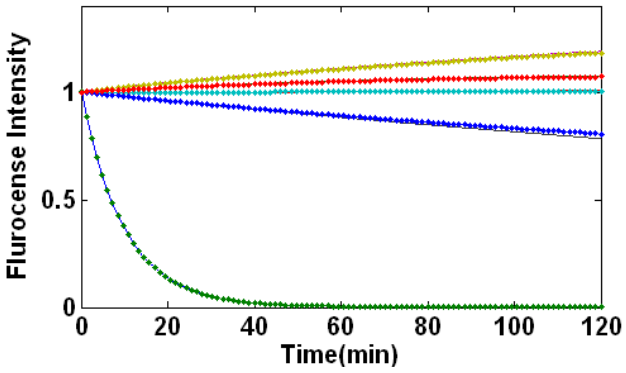

Fig.2

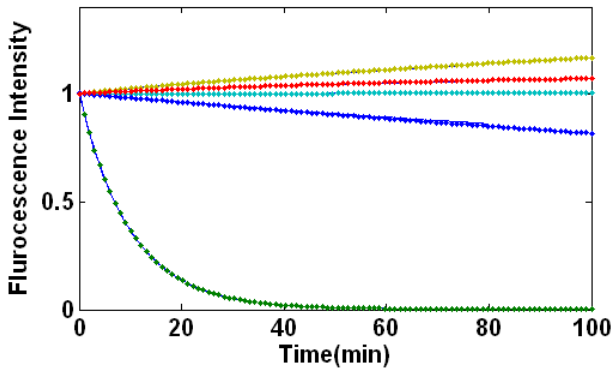

Fig.3

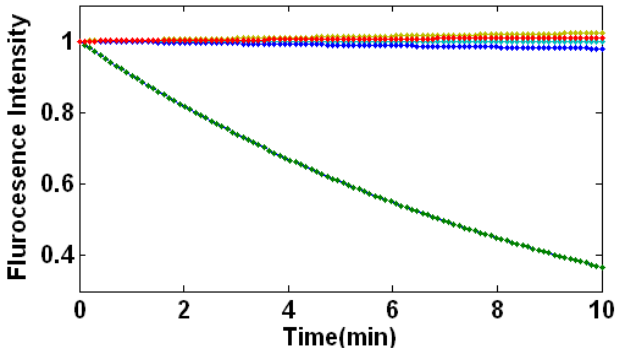

Fig.4

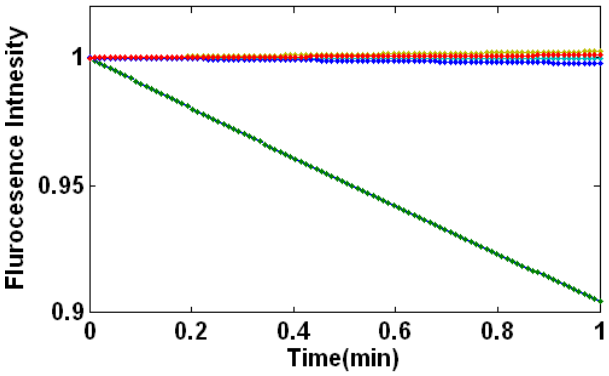

Fig.1-4. . Plot of dynamic response of transcriptional reporters such as a) Auxin b)TIRI c) [Auxin.TIRI] d) [Auxin.TIRI.VENUS] e) VENUS from bottom to top respectively versus time intervals in minutes using the equation(2). Solid line represents the numerical results where as dotted line exhibits the analytical results.

Fig.5

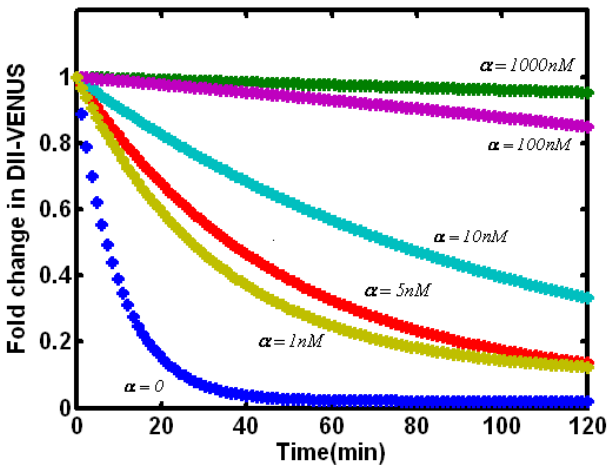

Fig..5 Plot of time versus fold changes in DII-VENUS signal for
$\alpha = 0, \alpha = 1\text{nM}, \alpha = 5\text{nM}, \alpha = 10\text{nM}, \alpha = 100\text{nM}, \alpha = 1000\text{nM}$
from bottom to top respectively using equation (2)

Fig..6

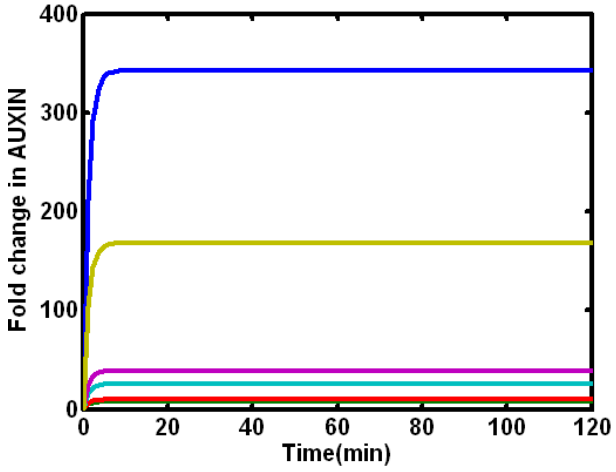

Fig.6. Plot of time versus the fold changes in auxin influx for various levels of external application of the auxin doses in the tissues of the plant cell such as $\alpha_1 = 5.83, \alpha_2 = 7.44, \alpha_3 = 19.96, \alpha_4 = 30.50, \alpha_5 = 132.82, \alpha_6 = 270.52$ using equation $X_1(t)$ in (2).

The parameters involved in this reaction are given by $k_a = 0.00082,\ k_d = 0.33, I_a = 1.15, I_d = 4.49,$ $I_m = 0.18, \delta = 0.49, \mu = 0.79, \lambda = 0.0032$

Nomenclature:

S.No	Parameter	Description
1	k_a	Rate of dissociation between auxin and TIRI/AFB
2	k_d	Rate of dissociation of the auxin-TIRI/AFB complexes
3	l_a	Rate of association of DII-VENUS to auxin-TIRI/AFB
4	l_d	Rate of dissociation of the auxin-TIRI/AFB-VENUS complexes into DII-VENUS and auxin-TIRI/AFB
5	l_m	Rate of dissociation of the auxin-TIRI/AFB-VENUS complexes into ubiquitinated DII-VENUS and auxin-TIRI/AFB
6	δ	Rate of DII-VENUS production
7	α	Rate of auxin influx
8	μ	Rate of auxin degradation and efflux
9	λ	Rate of decay of the DII-VENUS signal due to photobleaching
10	[TIRI]	Total concentration of TIRI/AFB receptors

ACKNOWLEDGEMENT

This work was supported by the CSIR (No.01(2422)/10/EMR-II.New Delhi, India. The authors are thankful to Dr. Murali, The Prinicpal, Madura college, Madurai and The Secretary, The Madura college Board, Madurai for their encouragement.

REFERENCES

[1] Xiaoyi Shan, JianbinYan and DaoxinXie, "Comparison of phytohormone signaling mechanisms",Current opinion in plant biology,2011,volume 15 Issue 1,84-91.
[2] J.J.Blakeslee, W.A.Peer, and A.S.Murphy.(2005). "Auxin transport".Current Opinion in Plant Biology.", 8, 494–500.
[3] S. Vanneste, J.Friml, "Auxin: a trigger for change in plant development",Cell,2009,136(6) ,1005-16,
[4] N. Dharmasiri, S,Dharmasiri, M.Estelle (2005), "The F-box protein TIR1 is an auxin receptor", Nature ,435:441–445.
[5] S,.Kepinski , O .Leyser (2005) ,"The Arabidopsis F-box protein TIR1 is an auxinreceptor.Nature ",435:446–451.
[6] X .Tan, et al. (2007)," Mechanism of auxin perception by the TIR1 ubiquitin ligase.Nature", 446:640–645.
[7] J.A. Ramos, N..Zenser., O.Leyser, J.Callis (2001) ,"Rapid degradation of auxin/indoleacetic acid proteins requires conserved amino acids of domain II and is proteasome dependent",Plant Cell 13:2349–2360.
[8] N .Zenser, A. Ellsmore, C.Leasure , J.Callis (2001) ,"Auxin modulates the degradation rate of Aux/IAA proteins",. ProcNatlAcadSci USA 98:11795–11800.
[9] K.A. Dreher, J. Brown , R.E. Saw, J. .Callis (2006), "The Arabidopsis Aux/IAA protein family has diversified in degradation and auxin responsiveness",. Plant Cell 18:699–714.
[10] L.Band,et al. (2012), "Root gravitropism is regulated by a transient lateral auxin gradient controlled by a tipping point mechanism, Proc.Natl. Acad. Sci. U.S.A. 109, 4668–4673
[11] V. Ananthaswamy, L. Rajendran, "Analytical solution of two-point non-linear boundaryvalue problems in a porous catalystparticles", International Journal of Mathematical Archive Vol. 3 (3), 2012, pp. 810-821.
[12] V. Ananthaswamy ,L. Rajendran, " Analytical solutions of some two-point non-linear elliptic boundary value problems", Applied Mathematics, Vol.3, 2012, pp. 1044-1058.
[13] V. Ananthaswamy ,L. Rajendran, " Analytical solution of non-isothermal diffusion- reaction processes and effectiveness factors", Article ID 487240, Vol. 2012, 2012, pp.1-14.
[14] V. Ananthaswamy, SP. Ganesan , L. Rajendran, " Approximate analytical solution of non-linear boundary value problem of steady state flow of a liquid film: Homotopy perturbation method", International Journal of Applied Science and Engineering Research (IJASER), Vol. 2 (5), 2013, pp. 569-577

APPENDIX A

Basic concept of Homotopy perturbation method [9-22]
To explain this method, let us consider the following function:

$$D_o(u) - f(r) = 0, \quad r \in \Omega \qquad \text{(A. 1)}$$

with the boundary conditions of

$$B_o(u, \frac{\partial u}{\partial n}) = 0, \qquad r \in \Gamma \qquad \text{(A. 2)}$$

where D_o is a general differential operator, B_o is a boundary operator, $f(r)$ is a known analytical function and Γ is the boundary of the domain Ω. In general, the operator D_o can be divided into a linear part L and a non-linear part N. Eq. (A. 1) can therefore be written as

$$L(u) + N(u) - f(r) = 0 \qquad \text{(A. 3)}$$

By the homotopy technique we construct a homotopy $v(r,p) : \Omega \times [0,1] \to \Re$ that satisfies

$$H(v,p) = (1-p)[L(v) - L(u_0)] + p[D_o(v) - f(r)] = 0. \qquad \text{(A. 4)}$$

$$H(v,p) = L(v) - L(u_0) + pL(u_0) + p[N(v) - f(r)] = 0. \qquad \text{(A. 5)}$$

where $p \in [0, 1]$ is an embedding parameter, and u_0 is an initial approximation of Eq. (A. 1) that satisfies the boundary conditions. From Eq. (A. 4) and Eq. (A. 5), we have

$$H(v,0) = L(v) - L(u_0) = 0 \qquad \text{(A. 6)}$$

$$H(v,1) = D_o(v) - f(r) = 0 \qquad \text{(A. 7)}$$

When p=0, Eq. (A. 4) and Eq. (A. 5) become linear equations. When p =1, they become non-linear equations. The process of changing p from zero to unity is that of $L(v) - L(u_0) = 0$ to $D_o(v) - f(r) = 0$. We first use the embedding parameter p as a "small parameter" and assume that the solutions of Eq. (A. 4) and Eq. (A. 5) can be written as a power series in p :

$$v = v_0 + pv_1 + p^2 v_2 + \ldots \qquad \text{(A. 8)}$$

Setting $p = 1$ results in the approximate solution of Eq. (A. 1):

$$u = \lim_{p \to 1} v = v_0 + v_1 + v_2 + \ldots \qquad \text{(A. 9)}$$

This is the basic idea of the HPM.

Appendix B

Matlab program for numerical solution of equation(1)

```
function
options= odeset('RelTol',1e-6,'Stats','on');
%initial conditions
x0 = [1; 1; 1; 1; 1];
tspan = [0,120];
tic
[t,x] = ode45(@TestFunction,tspan,x0,options);
toc
figure
hold on
plot(t, x(:,1))
plot(t, x(:,2))
plot(t, x(:,3))
plot(t, x(:,4))
plot(t, x(:,5))
legend('x','y','z')
ylabel('x')
xlabel('t')
return
function [dx_dt]= TestFunction(t,x)
ka=.00082;kd=0.33;la=1.15;Id=4.49;Im=0.18;;d=.49;s=0.
79;u=0.0032;
dx_dt(1)=kd*x(3)-ka*x(1)*x(2)+a-u*x(1);
dx_dt(2)=-ka*x(1)*x(2)+kd*x(3);
dx_dt(3)=ka*x(1)*x(2)-kd*x(3)+(Id+I1)*x(4)-
Ia*x(3)*x(5);
dx_dt(4)=Ia*x(3)*x(5)-(Id+I1)*x(4);
dx_dt(5)=d-Ia*x(5)*x(3)+Id*x(4)-s*x(5);
dx_dt = dx_dt';
return
```