Asymptotic Analysis of the Wnt/β Signaling Pathway

D T Maris and D A Goussis
Department of Mechanics, School of Applied Mathematics and Physical Sciences, National Technical University of Athens, 157 73 Athens, Greece
E-mail: dmaris@mail.ntua.gr, dagoussi@mail.ntua.gr

Abstract. The Wnt/β-catenin pathway is a signal transduction pathway made of proteins, which plays an important role in oncogenesis. Ethan Lee and co-workers introduced in 2003 a detailed mathematical model of this pathway, incorporating the kinetics of protein-protein interactions, protein synthesis/degradation and phosphorylation/dephosphorylation. The fast/slow dynamics of Lee's system are examined here, by employing the Computational Singular Perturbation (CSP) algorithm. CSP reproduces the results of the classical singular perturbation analysis in an algorithmic fashion, producing an approximation of (i) the low dimensional Slow Invariant Manifold (SIM), where the solution evolves and (ii) the reduced model that governs the flow there. The temporal variation of the dimensions of the SIM will be presented and the components of the pathway that are responsible (i) for the generation of the SIM and (ii) for driving the system on it will be identified.

1. Introduction
Models representing multiscale biochemical networks are usually non-linear and complex, making the interpretation of their dynamics a difficult task [1]. Among others, due to the generated equilibria between the fast components of such models, their long term evolution is determined by the slower components of the system [2]. The identification of these components is a very important step, since they generate and regulate the long term dynamics and response of the model.

Here, the most significant components of the Wnt signaling pathway model, that was introduced by Lee et al. in Ref. [3], will be identified. The Wnt signalling pathway is well known in the biology community due to its involvement to differentiation and to tissue development. It plays an important role to some kinds of cancers, such as colorectal cancer [4, 5] and in stem cell maintenance [5, 6]. The Wnt pathway is hard to model due two lack of quantitative experimental data, and most of the mathematical modeling research is based upon the detailed model [3]. It has been analyzed extensively, among others by studying its robustness [7, 8] and by constructing reduced models on the basis of four Partial Equilibrium approximations [9].

The detailed model of Lee et al. will be considered here and it will be shown here that is amenable to further reduction, beyond the four Partial Equilibrium approximations employed in [3]. The number of the new equilibria that develop will be identified, as the system evolves in time, along with the components of the system that are responsible for their development. These identifications will be based on the Computational Singular Perturbation (CSP) algorithm, which reproduces the results of the classical singular perturbation analysis [10, 11]. A brief presentation of CSP will be given first and the discussion of the results will follow.
2. The Computational Singular Perturbation method

Consider a system of \( N \) nonlinear ordinary differential equations (ODE’s):

\[
\frac{dy}{dt} = g(y) = S_1 R^1(y) + \ldots + S_K R^K(y)
\]

where \( y \) is the \( N \)-dimensional state vector and \( g(y) \) is the \( N \)-dimensional vector field. It is assumed that each of the \( K \) additive terms \( S_k R^k \) correspond to a distinct component of the physical process; e.g., in a kinetic pathway \( S_k \) is the \( N \)-dimensional stoichiometric vector of the \( k \)-th reaction and \( R^k \) the corresponding rate. Suppose that Eq. (1) exhibits \( M \) time scales, which are dissipative (tend to drive the system to equilibrium) and much faster than the rest. Geometrical Singular Perturbation Theory proves that under the action of these \( M \)-fast time scales the solution is attracted on a \((N-M)\)-dimensional SIM, where it evolves according to the slow time scales [12, 13]. Equation (1) can then be cast in the CSP form:

\[
\frac{dy}{dt} = a_r f^r + a_s f^s
\]

where \( a_r = (a_1, a_2, \ldots, a_M) \) and \( a_s = (a_{M+1}, a_{M+2}, \ldots, a_N) \) are the CSP vectors which span the \( M \)-fast and the \((N-M)\)-slow subspace of the tangent space, respectively. The amplitudes \( f^r = b^r g \) and \( f^s = b^s g \) are computed using the dual CSP vectors \( b^r = (b_1, b_2, \ldots, b_M)^T \) and \( b^s = (b_1^{M+1}, b_1^{M+2}, \ldots, b_1^N)^T \), where \( b^r \cdot a_j = \delta_{ij} \). When the \( M \)-fast time scales become exhausted the vector field \( g(y) \) lies entirely on the slow subspace, having no component in the fast one:

\[
f^r(y) \approx 0^r \quad \frac{dy}{dt} \approx a_s f^s
\]

The CSP basis vectors \( a_r \) and \( a_s \) along with their duals \( b^r \) and \( b^s \) are approximated by two iterative procedures; (i) the \( b^r \)-refinement, which improves the accuracy of \( a_s \) and \( b^r \) vectors and (ii) the \( a_r \)-refinement, which improves the accuracy of the \( a_r \) and \( b^s \) vectors. One \( a_r \)-refinement assures stability of the reduced system in Eq. (3) and more than one \( b^r \)-refinements improve the accuracy of the approximation of the SIM and of the solution from the reduced system [11, 14].

The construction of the reduced system allows for the acquisition of significant physical understanding of complex biological networks by employing the various CSP tools [2, 15]. For example, on the SIM where the \( M \)-fast time scales are exhausted, \( M \) equilibria develop among the various components of the system. Also, a number \((\geq M)\) of variables relate to these \( M \) fast time scales. These components and variables are identified by the CSP Participation Index \( P^m_k \) and CSP Pointer \( D_m \) defined as:

\[
P^m_k = \frac{\sum_{j=1}^K |f^m_j(y)|}{\sum_{j=1}^K |f^m_j(y)|} \quad D_m = \text{diag}[a_m b^m]
\]

where \( m = 1, \ldots, M \) denotes the corresponding fast CSP mode. By definition \(|P^m_1| + \ldots + |P^m_K| = 1\) and \(D^1_m + \ldots + D^K_m = 1 [16]\). A relatively large value of \( P^m_k \) denotes significant contribution of the \( k \)-th component to the \( m \)-th equilibrium and a relatively large \( D^m_m \) denotes strong relation of the \( n \)-th variable to the \( m \)-th fast time scale.

3. The Wnt model

As it is shown in Fig.(1), the Wnt pathway includes 17 reactions (steps) and 15 proteins and protein complexes (species). The most important features of this model are the core complexes participating in \( \beta \)-catenin phosphorylation and destruction. Except from \( \beta \)-catenin, these complexes include GSK3\( \beta \), APC and Axin. The steps that assemble or decompose these complexes are the forward reactions 4-10. Some steps of the pathway are presented: (a) the synthesis of Axin and of \( \beta \)-catenin (reactions 14 and 12), (b) the degradation of Axin (reaction 15), (c) the Axin-dependent degradation of \( \beta \)-catenin (reaction 13), and (d) the steps participating in the Destruction Core Cycle in order for phosphorylated \( \beta \)-catenin to be degraded (reactions 8, 9, 10, 11) [3].
The reversible binding steps involving APC (step 7), Axin (step 8), TCF (step 16) and β-catenin (step 17) along with their forward steps are considered very fast, so that the Partial Equilibrium approximation is valid [3]. Application of these four approximation yields a 15 dimensional ODE system, where rate equations in partial equilibrium are expressed through the remaining rates $V_4 - V_6$, $V_9$ and $V_{12} - V_{15}$. A complete list of the ODE system and the rate equations can be viewed in Lee et al. [3].

Table 1. Rates in Partial Equilibrium (left) and the existing conservation equations (right). $X_i, i = 1, ..., 15$ are the concentrations of proteins and protein complexes of the model and $V_j, j = 1, ..., 17$ are the rate equations. Concentrations marked with $^o$ are total (constants). Notation as in Lee et al [3].

| Rate Equation | Conservation Equation |
|---------------|-----------------------|
| $V_7f - V_{7b} \approx 0$ | $\beta$-catenin/*APC*/Axin*/GSK3 = $\beta$-catenin*/APC*/Axin*/GSK3 |
| $V_8f - V_{8b} \approx 0$ | $\beta$-catenin/*APC*/Axin*/GSK3 = $\beta$-catenin*/APC*/Axin*/GSK3 |
| $V_{16f} - V_{16b} \approx 0$ | $\beta$-catenin*/APC*/Axin*/GSK3 = $\beta$-catenin*/APC*/Axin*/GSK3 |
| $V_{17f} - V_{17b} \approx 0$ | $\beta$-catenin*/APC*/Axin*/GSK3 = $\beta$-catenin*/APC*/Axin*/GSK3 |

4. Reactions contributing to the formation of the SIM

Here, the case without Wnt stimulation ($Wnt = 0$) will be examined, so that the rates $V_1$, $V_2$ and $V_3$ are inactive and the $X_1$ and $X_2$ species do not participate. The results with Wnt stimulation are extracted with the same manner. The 15 dimensional Wnt model with 4 conservation equations and 4 Partial Equilibrium approximations, see Table (1), was analyzed with the CSP algorithm. Table (2) lists in column 3 the proteins and protein complexes that are identified by the CSP Pointer at a point in time where $M = 4$, see Fig.(2). The influence of the 7 reactions in the formation of the 8-dimensional SIM is assessed by the reaction in column 4 that were identified by the Participation Index (PI).

(a) $M = 1$: the pointer identifies mainly $\beta$-catenin*/APC*/Axin*/GSK3 and the PI suggests that reactions $V_9$ and $V_{10}$ equilibrate, so that $k_{10}X_9 = V_{10} \approx V_{11} = k_{11}X_{10}$. We can assume that it is amenable to Quasi Steady State approximation $V_9 \approx 0 = V_{10} \approx V_4 = V_5$.

(b) $M = 2$: the pointer identifies mainly (APC/Axin) and the PI indicates that $V_6f$ and $V_{6b}$ equilibrate, so that $k_6X_5X_6 = V_{6f} \approx 10V_{6b} = k_6X_4$. We can assume that the PEA is valid.

(c) $M = 3$: the pointer identifies $\beta$-catenin* and the PI indicates that there is an equilibria between reactions $V_{10}$ and $V_{11}$ so that $k_{10}X_9 = V_{10} \approx V_{11} = k_{11}X_{10}$. We can assume that $\beta$-catenin* is amenable to QSSA, $V_9 \approx 0 = V_{10} \approx V_4 \approx V_5$.

(d) $M = 4$: the pointer identifies mainly (APC/Axin/GSK3) and the PI produces the equilibration $k_{4}X_4 = V_4 \approx V_5 = k_5X_4$, given the equilibration $V_{6b} \approx V_{6b}$ and that $V_3 = 0$ we can assume that (APC/Axin/GSK3) is amenable to QSSA, $V_3 \approx V_4 + V_5 + V_6$.
The evolution of the seven bounded time scales of the modified model are examined and it is shown that the number of fast time scales $M$ increases with time; the time scales $\tau_i, i = 1, \ldots, 7$ are denoted in black; $M$ (blue) shows the increase of the exhausted time scales with time for one one a$_r$-refinement and one b$_r$-refinement.

Table 2. Proteins and protein complexes indicated by CSP Pointer are shown in column 3 (largest pointed marked bold); reactions exhibiting Participation Indices greater than 5% are listed in column 4 (those providing PI’s greater than 15% are marked by bold); reactions with + sign equilibrate with those with the - sign.

| M | Amplitude of exhausted modes | Proteins and protein complexes identified by CSP Pointer | Reactions |
|---|-------------------------------|------------------------------------------------------|------------|
| 1 | $f'$ | (β-catenin*/APC*/Axin*/GSK3). (β-catenin/APC*/Axin*/GSK3) | 9. -10 |
| 2 | $f'^2$ | (APC/Axin). (APC*/Axin*/GSK3), Axin | -6f. 6b |
| 3 | $f''$ | β-catenin* | 10. -11 |
| 4 | $f'^4$ | (APC/Axin/GSK3), (APC*/Axin*/GSK3) | -4. 5.-6f. 6b |

5. Conclusions
The system of ODE’s by Lee et al. exhibits multiscale behavior and it has amenable to singular perturbation analysis. It was demonstrated here that the algorithmic methodology of CSP can readily be employed, without the need (i) to cast the system in the proper non-dimensional form mechanism and (ii) to find the proper non-dimensional small parameters. The underlying physical insight for the control of the system was thus obtained without any effort. It was shown that as time evolves there exist 4 fast modes, see Fig 2. The fastest mode relates to the equilibration of the phosphorylation of β-catenin and its release through the destruction complex. The second mode relates to (APC/Axin) and the equilibria that forms between binding and releasing GSK3. The third mode involving β-catenin* indicates that the rate β-catenin* polyubiquitinated is the same as it is released from the destruction complex. The last mode involves the protein complex (APC/Axin/GSK3). Given the equilibria that forms when (APC/Axin) binds and releases GSK3, an equilibrium develops between the phosphorylation and dephosphorylation of APC and Axin in the (APC/Axin/GSK3) complex.

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7. References
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