

Computational Modeling of Cell Survival/Death Using BiCMOS

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Abstract—A well-structured and controlled design methodology, along with a supporting hierarchical design system, has been developed to optimally support the development effort on several programs requiring gate array and semi custom Very Large Scale Integration (VLSI) design. In this paper, we will present an application of VLSI in System Biology. This work examines signaling networks that control the survival decision treated with combinations of three primary signals the pro death cytokine, tumor necrosis factor- α (TNF), and the pro survival growth factors, epidermal growth factor (EGF) and insulin. We have made model by taking three inputs, than made the truth table, Boolean equation and than implement the equation using BiCMOS logic circuit.

Index Terms—Tumor Necrosis Factor- α , Epidermal Growth Factor, Insulin, BiCMOS

I. INTRODUCTION

Very Large Scale Integration (VLSI) is the field, which involves packing more and more logic devices into smaller and smaller areas. The complexity of VLSIs being designed and used today makes the manual approach to design impractical. Our aim is to use VLSI in System Biology. Computational systems biology addresses questions fundamental to our understanding of life, yet progress here will lead to practical innovations in medicine, drug discovery and engineering. Substantial progresses over the past three decades in biochemistry, molecular biology and cell physiology, coupled with emerging high throughput techniques for detecting protein-protein interaction, have ushered in a new era in signal transduction research. Cell signaling pathways interact with one another to form networks. Such networks are complex in their organization and exhibit emergent properties such as bistability and ultrasensitivity [1]. To understand complex biological systems requires the integration of experimental and computational research - in other words systems biology approach. This work examines signaling networks that control the survival decision treated with combinations of three primary signals [2, 3] the pro death cytokine, *tumor necrosis factor- α* (TNF) [4, 5], and the pro survival growth factors, *epidermal growth factor* (EGF) [6, 7] and insulin [8, 9, 10]. The system output is typically a phenotypic readout (death or survival); however, it can also be determined by measuring “early” signals that perfectly correlate with the death/ survival output. Examples of such early signals

include phosphatidyserine exposure, membrane permeability, nuclear fragmentation and caspase substrate cleavage. We have implemented the signaling system heading by three input signals such as TNF, EGF and insulin.

II. COMPUTATIONAL MODEL

The prediction model for cell death/survival has been implemented using SPICE. We have implemented the signaling system heading by three input signals such as TNF, EGF and insulin. The block diagram of the signaling system that was modeled is shown in Figure 1.

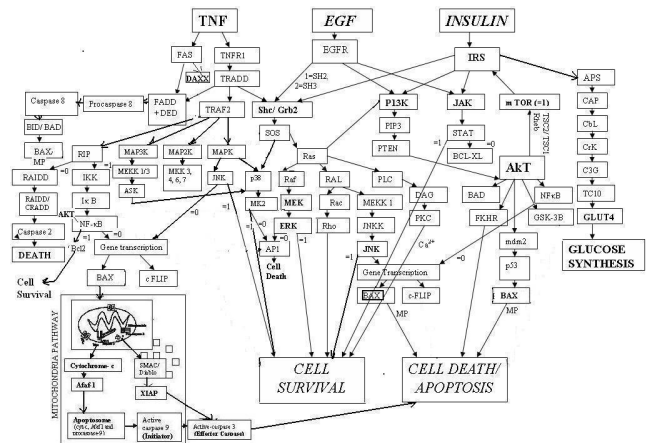


Figure 1 Computational Model for cell survival/ death

A. Tumor Necrosis Factor- α (TNF):

There are two receptors, **TNF-R1** (TNF receptor type 1) and **TNF-R2** (TNF receptor type 2), bind to TNF [4]. TNF-R1 is constitutively expressed in most tissues, and can be fully activated by both the membrane-bound and soluble trimeric forms of TNF, while TNF-R2 is only found in cells of the immune system and respond to the membrane-bound form of the TNF homotrimer. As most information regarding TNF signaling is derived from TNF-R1, the role of TNF-R2 is likely underestimated. Upon contact with their ligand, TNF receptors also form trimers, their tips fitting into the grooves formed between TNF monomers. This binding causes a conformational change to occur in the receptor, leading to the dissociation of the inhibitory protein SODD from the intracellular death domain. This dissociation enables the adaptor protein TRADD to bind to the death domain, serving as a platform for subsequent protein binding. TNF receptor associated factor 2 (TRAF2) is a prototypical member of the TRAF family proteins that regulates signals from the TNF receptors [11], resulting in sequential activation of MAP3K (MEKK1/3, ASK1/2) [12],

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MAP2K (MKK3, 4, 6, 7) [13] and MAPK (JNK, p38) [14], as well as in activation of RIP/IKK signaling pathways. MAPK and IKK in turn activate AP-1 and NF- κ B transcription factors. Activation of AP-1 and NF- κ B induces genes involved in inflammation, immune response, cell proliferation and cell differentiation, as well as genes that act to suppress death receptor- and stress-induced apoptosis. The signaling pathways from RIP/IKK to NF- κ B and from MAP3K to AP-1 are better understood, the receptor proximal events that determine TRAF2-dependent activation of RIP/IKK vs. MAP3K remain largely elusive shown in Figure 1.

Induction of death signaling: Like all death-domain containing members of the TNFR superfamily, TNF-R1 is involved in death signaling. However, TNF-induced cell death plays only a minor role compared to its overwhelming functions in the inflammatory process. Its death inducing capability is weak compared to other family members (such as Fas), and often masked by the anti-apoptotic effects of NF- κ B. Nevertheless, TRADD binds FADD, which then recruits the cysteine protease caspase 8 [15]. A high concentration of caspase 8 induces its autoproteolytic activation and subsequent cleaving of effector caspases, leading to cell apoptosis [16, 17]. Cell death is an essential strategy for the control of the dynamic balance in living systems, and two fundamentally different forms of cell death, *apoptosis and necrosis* [18], have been defined.

B. Epidermal Growth Factor (EGF) :

Upon ligand-binding receptors homo-dimerise or hetero-dimerise triggering tyrosine [19] trans-phosphorylation of the receptor sub-units. Intracellular tyrosine kinases of the Src family and Abl family are also able to tyrosine phosphorylate ErbB receptors. These tyrosine phosphorylated sites allow proteins to bind through their Src homology 2 (SH2) domains leading to the activation of downstream signaling cascades including the RAS/extracellular signal regulated kinase (ERK) pathway, the phosphatidylinositol 3 kinase (PI3K) pathway and the Janus kinase/Signal transducer and activator of transcription (JAK/ STAT) pathway. Differences in the C-terminal domains of the ErbB receptors govern the exact second messenger cascades that are elicited conferring signaling specificity. The EGF signal is terminated primarily through endocytosis of the receptor-ligand complex. A number of signal transduction pathways branch out from the receptor signalling complex as shown in Figure 1.

EGF activates the ERK pathway through the binding of Grb2 or Shc to phosphorylated ErbB receptors, which in turn results in the recruitment of the son of sevenless (SOS) to the activated receptor. SOS then activates RAS leading to the activation of RAF 1 [14, 20]. RAF-1 subsequently phosphorylates MEK1 and MEK2 which activate respectively ERK 1 and ERK2.

MAP kinases are actually a family of protein kinases that are widely distributed and are found in all eukaryotic organisms. These can be classified into three main functional groups [12, 14]. The ERK pathway responds to mitogen activation. In the JNK/SAPK pathway SAPK stands for stress activation protein kinase and within this class of kinases the Jun N-terminal kinases (JNK) for a

subfamily. In the p38/HOG pathway HOG stands for high osmolarity glycerol where the p38 proteins are a subfamily.

EGF also promotes cell survival through the activation of PI3 kinase/Akt signaling [1, 2, 3]. EGF triggers the recruitment of PI3 kinase to activated ErbB receptors, which is mediated by the binding of SH2 domains in PI3-kinase to phosphorylated tyrosine residues. PI3-kinase can also activate RAS, resulting in the activation of ERK signaling, thereby facilitating cross-talk between survival pathways. A key downstream effector of PtdIns(3,4,5)P₃ is AKT(PKB). AKT promotes cell survival through the transcription of anti-apoptotic proteins [21]. Intermediate transcription factors involved in this process are NF κ B and CREB. Another downstream target of AKT is glycogen synthase kinase 3 (GSK3). Under basal conditions the constitutive activity of GSK3 leads to the phosphorylation and inhibition of a guanine nucleotide exchange factor eIF2B, which regulates the initiation of protein translation. AKT also activates mammalian target of rapamycin (mTOR) [22], which promotes protein synthesis through p70 ribosomal S6 kinase (p70s6k) and inhibition of eIF-4E binding protein (4E-BP1).

Another signaling cascade initiated by EGF is the JAK/STAT pathway, which is also implicated in cell survival responses [23]. JAK phosphorylates STAT proteins localized at the plasma membrane. This leads to the translocation of STAT proteins to the nucleus where they activate the transcription of genes associated with cell survival

C. Insulin :

Activation of PI3K by insulin, insulin-like growth factor-1 resulted in a regulation of broad range of cellular functions. Akt (protein kinase B, c-Akt) is one of the serine/threonine kinases downstream of PI3K. Akt was originally implicated in cancer development, promoting cell proliferation and inhibition of apoptosis. Insulin and other growth factors acutely activate Akt. Once active, AKT enters the cytoplasm where it leads to the phosphorylation and inactivation of glycogen synthase kinase 3 (GSK3) leading to cell death.

Cell signaling mediated by G protein switch involves the tuberous sclerosis complex (TSC), tumor suppressors (TSC1 and TSC2) and the Ras-related small G protein Rheb. A complex between TSC1 and TSC2 is regulated by multi-site phosphorylation and acts as a point of integration for a diverse array of cellular signals, including those arising from growth factors, nutrients, and a variety of stress conditions. When active, the TSC1-TSC2 complex [24, 25] acts as a GTPase activating protein (GAP) for Rheb, thereby turning Rheb off by stimulating its intrinsic GTPase activity. In the presence of growth factors and nutrients, this complex is turned off, allowing the GTP-bound active version of Rheb to accumulate and turn on downstream pathways. The best-characterized downstream effectors of Rheb is the mammalian target of rapamycin complex 1 (mTORC1), a critical regulator of cell growth and proliferation.

III. RESULTS AND DISCUSSIONS

A known deficiency of MOS technology lies in the limited load drive capabilities of MOS transistors. This is due to the limited current sourcing and current sinking abilities associated with both p- and n- transistors and although it is possible, for example, to design supper buffers using MOS transistors alone, such arrangements do not always compare well with the capabilities of bipolar transistors. Bipolar transistors also provide higher gain and have generally better noise and high frequency characteristics than MOS transistors and use of BiCMOS gates may be an effective way of speeding up VLSI circuits. To take advantage of BiCMOS, the completely functional entity, not just the logic gate, must be considered. A comparison between the characteristics of CMOS and bipolar circuits is set out in Table and the differences are self-evident. BiCMOS technology goes some way toward combining the virtues of both technologies.

Above we had studied relating how TNF, EGF and Insulin work and its pathways in detail and explain each and every possible path for that. Based on pathways we had made truth tables for every possible path for cell survival/death. Than we realize the truth tables by Karnaugh Map (K-Map) and get the Boolean expression for its individual possible paths. We simulate the results of each path, then combine all the results, and simulate through SPICE simulator using BiCMOS, get result of TNF, EGF and Insulin for its cell survival/death. In output, '1' signifies cell survival and '0' signifies cell death. For cell survival the ten different proteins i.e. P13K, TNFR1, EGFR, IRS, IKK, Grb2, SOS, Ras, TRADD, Traf2 should present. If any one of them is absent than there is a cell death. In Figure 2, first three blocks shows the input and last block show the output of TNF. V(9), V(36) etc represents the output of each possible path used for TNF as cell survival/death. We get final output from V(49), Similarly, Figure 3, 4 and 5 shows the simulated result of EGF. Insulin and combination of three i.e. TNF, EGF and Insulin.

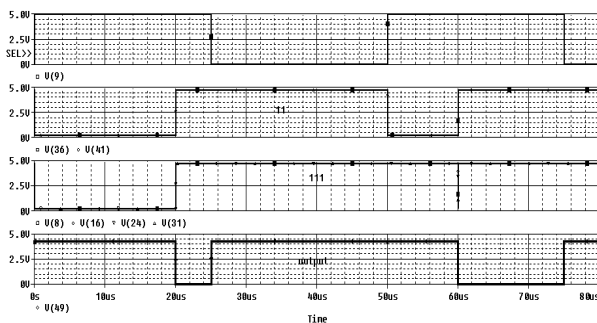


Figure 2 Output signals of cell survival/ death from SPICE simulation considering TNF as input

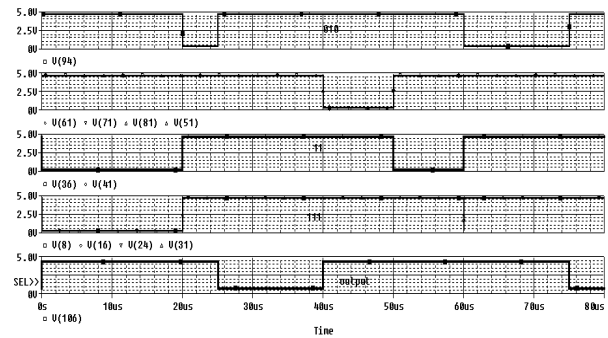


Figure 3 Output signals of cell survival/ death from SPICE simulation considering EGF as input.

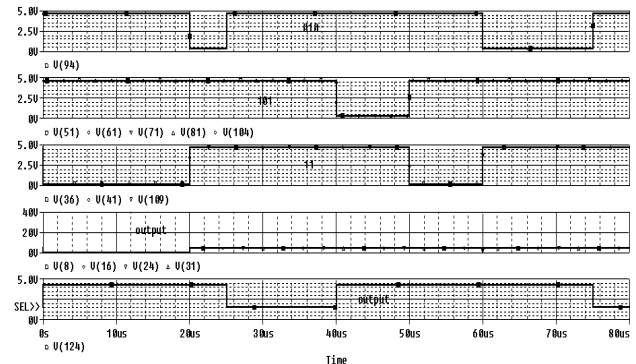


Figure 4 Output signals of cell survival/ death from SPICE simulation considering Insulin as input

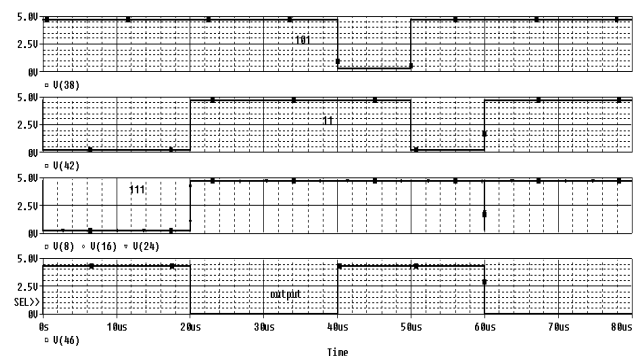


Figure 5 Output signals of cell survival/ death from SPICE simulation considering TNF, EGF and Insulin as input

IV. CONCLUSION

We had successfully made computational model for cell survival/ death using three inputs such as TNF, EGF and insulin. With that model we had made truth table, Boolean expression and logical circuit for each possible pathway. We then simulate the results of each path and then combine all the results and get result of TNF, EGF and Insulin for its survival/ death using BiCMOS logic circuit.

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ABBREVIATIONS

AP-1, Activation Protein 1; ASK1, Apoptosis signal-regulating kinase 1; EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; ERK, extracellular-regulated kinase; FADD, Fas-Associated protein with Death Domain; FKHR, Forkhead transcription factor; GLUT4, Glucose transport; Grb2, growth factor receptor-bound 2; GSK 3, Glycogen synthase kinase 3; HOG, High osmolarity glycerol; IGF, insulin-like growth factor; IκB, I Kappa B (nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor); IKK, IκB kinase; IR, insulin receptor; IRS1, insulin receptor substrate 1; JNK1, c-jun NH2 terminal kinase 1; MAP kinases, mitogen-activated protein kinases; MEK, mitogen-activated protein kinase and extracellular-regulated kinase kinase; MK2, mitogen-activated protein kinase-activated protein kinase 2; mTOR, mammalian target of rapamycin; NF-κB, nuclear factor-κB; PDK, Phi Delta Kappa; PI3K, phosphatidylinositol 3-kinase; p38, P38 mitogen-activated protein kinases; pT-EGFR, phospho-to-total EGFR; pT-Akt, phospho-to-total Akt; Rac, Ras-related C3 botulinum toxin substrate; SAPK/JNK, Stress-activated protein kinase/Jun-amino-terminal kinase; SH2, Src homology 2; SODD, Silencer of death domains; SOS, Son of Sevenless; TNF, tumor necrosis factor; TNFR1, tumor necrosis factor receptor 1; TNFR2, tumor necrosis factor receptor 2; TRADD, TNFR associated via death domain; TRAF2, TNF receptor associated factor 2, TSC, Tuberous sclerosis complex; XIAP, X-linked Inhibitor of Apoptosis Protein.