Title: A Stochastic Approach to Modeling NF-kB Pathway

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Introduction

The IκB-NF-κB signaling pathway is a cornerstone in the regulation of inflammation, immune response, and cellular fate across various cell types. This intricate circuit is triggered by the binding of diverse Toll-like receptors (TLRs) to pathogens or cytokines like TNF-α, initiating a cascade that liberates the NF-κB transcription factor from its inhibitor, IκB, in the cytoplasm. The freed NF-κB then migrates to the nucleus, orchestrating the expression of genes critical for immune function and cell survival. Crucially, NF-κB also upregulates IκB-α, an isoform of its inhibitor, creating a negative feedback loop that controls the intensity and duration of its own activity through a pattern of oscillations—crucial for maintaining cellular balance.

This dynamic network, a pivot between apoptosis and proliferation, is susceptible to dysregulation due to infections or genetic alterations, often culminating in chronic inflammation or cancer. Understanding the stochastic nature of this pathway is vital, given that cellular processes inherently exhibit randomness, which can significantly influence biological outcomes. Therefore, to enhance our understanding of the NF-κB pathway and its myriad biological roles, a systems biology approach is paramount—one that marries rigorous experimentation with sophisticated stochastic modeling.

This paper presents a simple model that builds upon the foundation laid by the original model. By employing a stochastic framework, we aim to capture the inherent variability and complex dynamics of the NF-κB signaling pathway, highlighting the critical role of probabilistic events in cellular decision-making processes. The research problem at hand is to delineate how stochastic elements influence the NF-κB pathway's regulatory mechanisms and to discern the implications of this variability on disease pathogenesis and cellular function. The paper will discuss the modeling assumptions, interpret the findings, and propose directions for future research, which are essential for devising targeted therapeutic strategies.

Literature Review

Biochemistry of NF-kB pathway

NF-kB is a family of transcription factors that have been found to be central to mammalian immune response. Although, initially found in B lymphocytes, they are present in a variety of cell types as regulators of cell growth, development and activation. It is considered a pleiotropic gene that codes for transcription factors, which can bind to promotor regions of a large number of genes responsible for regulating many normal cellular functions such as growth, proliferation, apoptosis, immune and inflammatory responses. Due to its involvement in such important pathways, its anti-apoptotic and pro-inflammatory functions have been found to be upregulated in cancer cells.

The mammalian NF-kB family is composed of five proteins: RelA (also known as p65), RelB, c-Rel, NF-kB1 (also known as p50), and NF-kB2 (also known as p52). The RHD (Rel homology domain) structurally differs among this family of proteins. This structural distinction translates into functional characteristics as all members of the family dimerize and contribute to regulation of NF-kB in different ways. A dozen types of dimers are possible to form, and their interplay initiates gene expression, its positive and negative regulation. Consequently, thresholds are set for expression of particular genes.

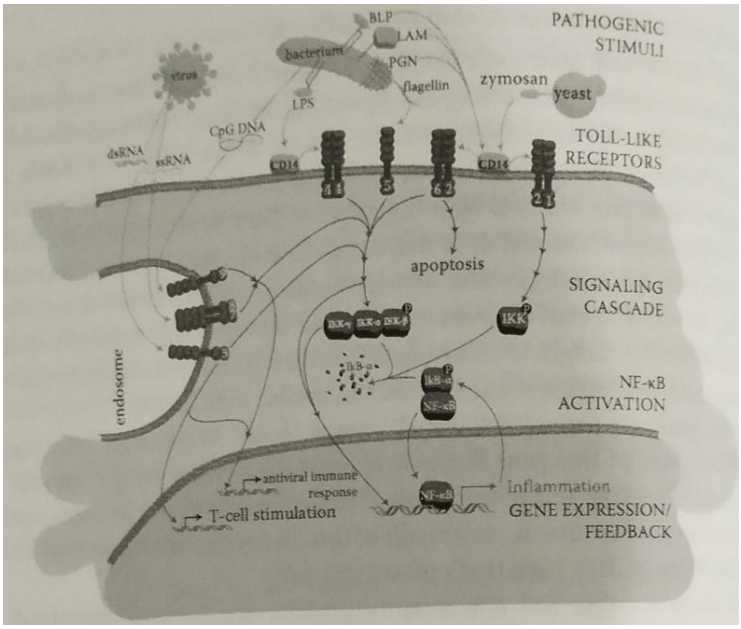

Fig 1: Schematic of NF-kB pathway (Fundamental of Systems Biology, by Markus W.Covert)

Contrasting with this family of NF-kB transcription factors are a family of inhibitors - IkBα, IkBβ and IkBϵ mainly. Ankyrin domains are shared by all members of the IkBs and are essential for certain interactions with the Rel-homology domain, found on NF-kB transcription factors. Under normal cellular conditions, NF-kB proteins are bound to the IkBs in the cytoplasm as the inhibitors

restrict the nuclear translocation and subsequent acivation of NF-kB. When cells are stimulated by inflammatory or immune responses via ligand binding of invading bacteria, cytokines, viruses, etc with TLRs (toll-like receptors, on cell membrane), the IkBs are phosphorylated by activated IKKs (IkB kinases). Subsequently, the IkBs undergo proteasome degradation and NF-kB transcription factors in the cytoplasm are free to translocate to the nucleus. NF-kB dimers bind to the promoters of many genes in the nucleus, activating transcription and translation of proteins that up-regulate immune and inflammatory responses. Additionally, NF-kB dimers also up-regulate protein synthesis of IkBα which can cause huge negative feedback for NF-kB. Similarly, IkBβ and IkBϵ are also synthesized but over longer periods of time relative to IkBα synthesis. Thus, all IkBs down-regulate NF-kB at varied times and over different durations.

Mathematical Modeling Intervention

Misregulation of this pathway is implicated in chronic inflammatory diseases and cancer. Mathematical modeling of the IkB-NF-kB signaling module has been pivotal in understanding the complex dynamics of this pathway. The original model, the Hoffman-Levchenko model involved a system of differential equations based on the kinetics of the association, dissociation, synthesis, degradation, and translocation of IKK, IkB, and NF-kB species. This model, and its subsequent refinements, have revealed the roles of different IkB isoforms in modulating NF-kB activity, the importance of negative feedback in controlling NF-kB dynamics, and the ability of the system to differentiate between short and long-lasting stimuli. The original model demonstrated that IκBα is in charge of strong negative feedback and the “rapid switch-off" of the NF-κB response, whilst IκBβ and −ϵ were in charge of minimizing oscillation potential in the system and stabilizing NF-κB responses in response to prolonged stimulations.

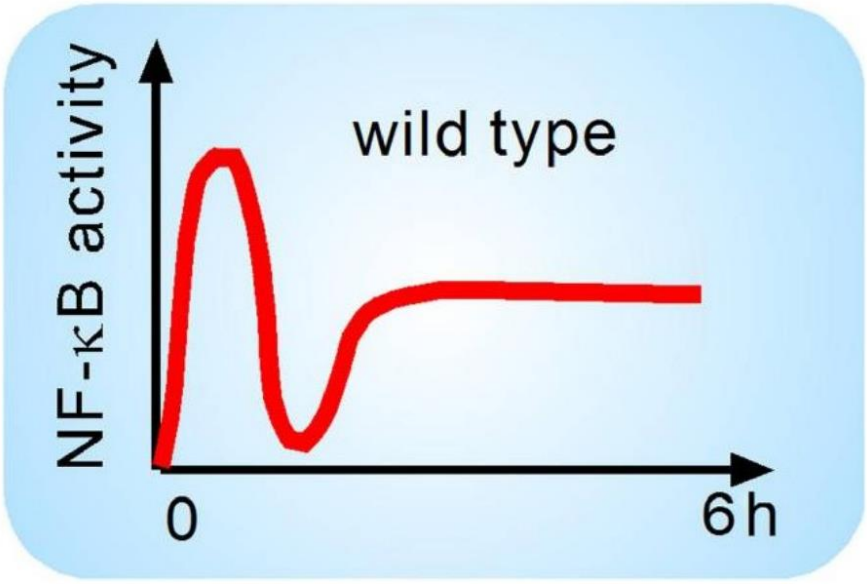

Fig 2: Bi-phasic behavior of NF-kB activity upon continuous stimulation in WT cells (all IkB isoforms are active); The dip is caused by IkBα while a level of activity is maintained by Ikβ and - ϵ activation (The IkappaB-NF-kappaB signaling module, Hoffman et al., 2002)

Subsequent studies have explored additional feedback mechanisms, such as the roles of IkBε and A20, and their effects on NF-kB activity. These studies have shown that feedback loops are crucial in controlling the temporal dynamics of NF-kB activity, thus regulating the expression of inflammatory genes. Moreover, the NF-kB pathway interacts with other signaling pathways,

indicating a complex network of crosstalk that influences its activity. For instance, different stimuli like LPS (lipopolysaccharide) and TNFα (tumor necrosis factor alpha) can activate NF-kB through distinct mechanisms, leading to different NF-kB dynamics and gene expression profiles. This crosstalk extends to non-inflammatory stimuli as well, further complicating the regulation of the pathway.

Mathematical models have also been employed in drug targeting studies, aiming to modulate NF-kB activity in diseases like arthritis, autoimmune disorders, and cancer. By simulating the effects of various inhibitors on NF-kB dynamics, these models help in understanding drug specificity and the potential effects of combining multiple drugs. In conclusion, mathematical modeling has been essential in unraveling the regulatory mechanisms of the NF-kB pathway, enhancing our understanding of its role in inflammation and disease. These models have not only provided insights into the pathway's dynamics but have also paved the way for targeted therapeutic interventions. The integration of these models with experimental data has been instrumental in advancing our knowledge of cellular signaling processes, particularly in the context of inflammation and immune activation.

Gaps in literature

In reviewing the mathematical models of the IκB-NF-κB signaling pathway detailed in the review: "Understanding NF-κB signaling via mathematical modeling", several gaps in the literature, especially regarding stochastic simulations, become evident. These gaps are crucial as they pertain to the inherent biochemical randomness and cellular variability, aspects not fully captured by deterministic models.

Firstly, a significant gap is the underrepresentation of stochasticity in modeling the dynamics of NF-κB activation and its regulation by IκB proteins. Deterministic models, while insightful, often fail to account for the heterogeneity observed in single-cell responses. NF-κB signaling exhibits notable cell-to-cell variability in response to stimuli, a phenomenon that stochastic models are better equipped to simulate. Incorporating stochastic elements would provide a more realistic representation of the pathway's behavior, especially under varying external conditions.

Another notable gap is the lack of stochastic models in understanding the crosstalk between NF-κB and other signaling pathways. Deterministic approaches, though prevalent, might oversimplify the interactions that contribute to the variability in cellular responses. Stochastic simulations could

offer insights into how these interactions influence the overall behavior of the signaling network, particularly in different pathological states.

Additionally, the spatial dynamics of NF-κB signaling, including the localization and translocation between the cytoplasm and nucleus, and the dynamics of IκB synthesis and degradation, are areas where stochastic elements are notably lacking. Spatial stochastic models could enhance our understanding of these processes, considering the complexity and heterogeneity of cellular environments.

Furthermore, while some existing models integrate stochastic elements, there is an evident need for more comprehensive, multi-scale stochastic models. These should encompass various aspects of the pathway, such as gene expression, protein modification, and cellular signaling processes. Such integrative compartment models would provide a more complete picture of the role of noise and variability in NF-κB signaling, shedding light on its biological implications.

In conclusion, the existing mathematical models of the IκB-NF-κB pathway, though foundational, reveal significant gaps in the context of stochastic simulations. Addressing these gaps by incorporating stochastic elements across different levels of the pathway is essential. This approach would not only enhance our understanding of the pathway's dynamics but also illuminate its role in diverse physiological and pathological contexts, potentially leading to more targeted therapeutic strategies.

In this report, a simple stochastic model of the NF-kB pathway is shown. The heterogeneity in the response of different cells to the same stimuli of inflammation is modelled with randomness accounted by the exact Gillespie algorithm.

Methodology

NF-kB pathway can occur in two ways: canonical/normal and non-canonical/alternate. Canonical pathway is triggered by inflammatory signaling such as TNFα receptor-ligand binding, cytokine binding, presence of immune cells, etc. In such a case, IKKβ is enough for ubiquitin-mediated proteasome degradation of IkBα and IkBβ while, IKKα regulates gene expression by phosphorylating histones in the nuclear DNA. The release of NF-kB proteins followed by degradation on IkBs, leads to the translocation of NF-kB proteins to the nucleus where they induce the expression of many genes including IkBs and A20. As mentioned in the earlier section, this leads to strong negative feedback from IkBα which inhibits NF-kB activity while A20 protein

inactivates IKK by catalyzing its transformation into IKKi (inactive form). Both the classic and alternative pathways are primarily activated during immune system function; however, the former is primarily involved in the inflammatory response and in controlling lymphoid cell proliferation and apoptosis during the immune reaction, while the latter controls lymphoid organogenesis and, as a result, its engagement primarily happens in response to non-inflammatory stimuli.

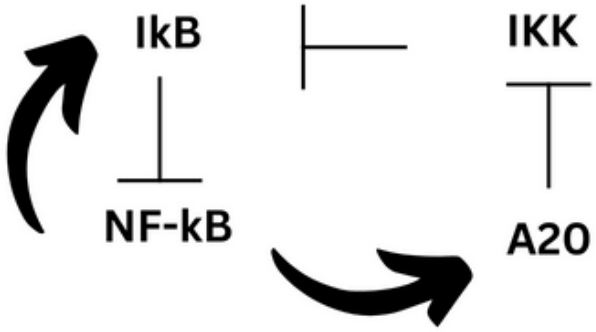

Fig 3: Regulatory network in canonical pathway, comprising of IkB, NF-kB, A20 and IKK species

After stimulation, NF-κB activity is typically visible ten minutes later, and certain NF-κB sensitive promoters are triggered almost instantly. As was previously noted, NF-κB activation results in the transcriptional upregulation of IκBα, which sets off a strong negative feedback loop that represses NF-κB. This mechanism's activity can be reactivated in response to another stimulus and may lead to a NF-κB activity oscillation pattern during prolonged stimulation.

As seen in Fig 4, a simple regulatory framework has been considered for the modelling of this complex NF-kB pathway. The model takes a simplifying assumption that the activators of the pathway are NF-kB, IKK while the inhibitors are IkB and A20 molecules. The NF-kB dimer (p50-p65) commonly found in the canonical pathway and IKK complex are considered as single proteins as the kinetics of the dimers are disregarded. Biochemically, IKK exists in active, inactive and neutral states but we are only considering an on-off switch where IKK is either active or inactive. The mRNA production induced by NF-kB is an indication for its activity or immune/inflammatory response. The production and degradation of mRNA have also been considered to keep the model physiologically relevant. The model starts with initial conditions when all the species are inactive.

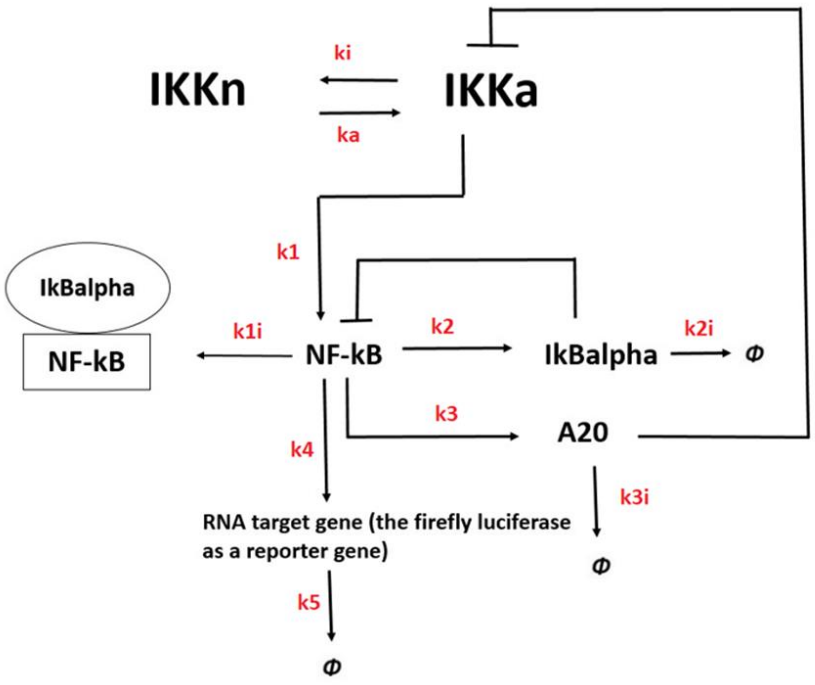

Fig 4: Sketch of the regulatory pathway considered for the model (Modeling and data analysis of biochemical oscillators using Chemical Master Equation and AI: applications to the NF-kB activity in patient derived xenografts, Manuela Carriero)

As seen in the table below, 10 rate constants are involved in the transitions of activation and inactivation of IKK, NF-kB, IkBα (only the isoform with strong negative feedback is considered) and mRNA production, degradation kinetics.

Transitions	**Rates**
IKK activation	k_a (IKKn)(1/(1+〖A20〗_active))
IKK inactivation	k_a (IKKa)(〖A20〗_active)
NF-kB activation	k1"(NF-kB:IkBα)(IKKa)"(1/(1+〖IKBα〗_active))

NF-kB inactivation	k1i(NFkB)(〖IkBα〗_active)
IkBα activation	k2(NFkB)〖IkBα〗_inactive
IkBα inactivation	k2i(IKKa)〖(〖IkBα〗_active)A20〗_active
A20 activation	k3(〖A20〗_inactive)NFkBa
A20 inactivation	k3i(〖A20〗_active)NFkBi
RNA production	k_4 NFkBa
RNA degradation	k_5 RNAs

Data Analysis

In this section, the results of simulations for the simple framework (Fig 4) are discussed. To model this system, R language's GillespieSSA2 package was utilized and all the simulations were made using exact Gillespie algorithm. As seen in Fig 5, the products of NF-kB pathway that is the mRNA transcribed is oscillating with time. Other species are not seen to oscillate with similar amplitudes as this is not a compartment model and this could be an implication of rate constant values considered. Figure 6 clearly shows these oscillations in products formed and this is due to the strong negative feedback by IkBα along with inhibition by A20.

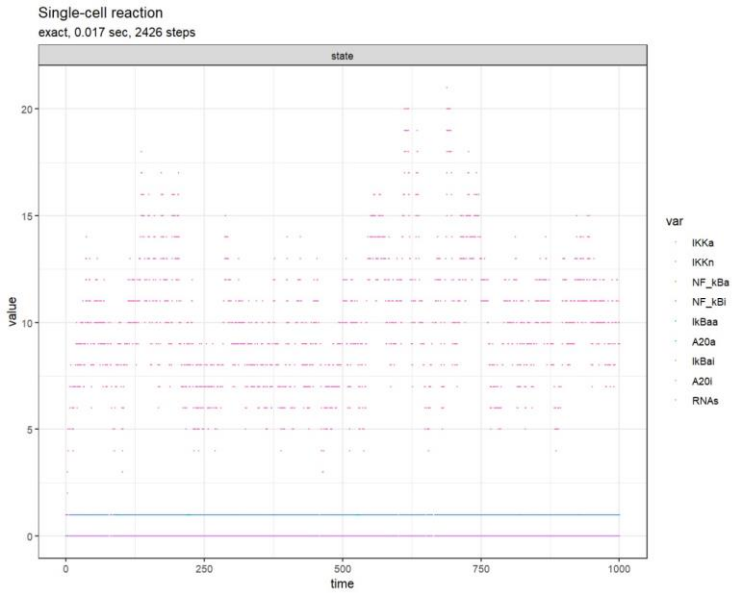

Fig 5: The dynamics of active and inactive forms of species: IKK, NF-kB, IkBα and A20 in a single reaction assumed to have initiated in a single cell

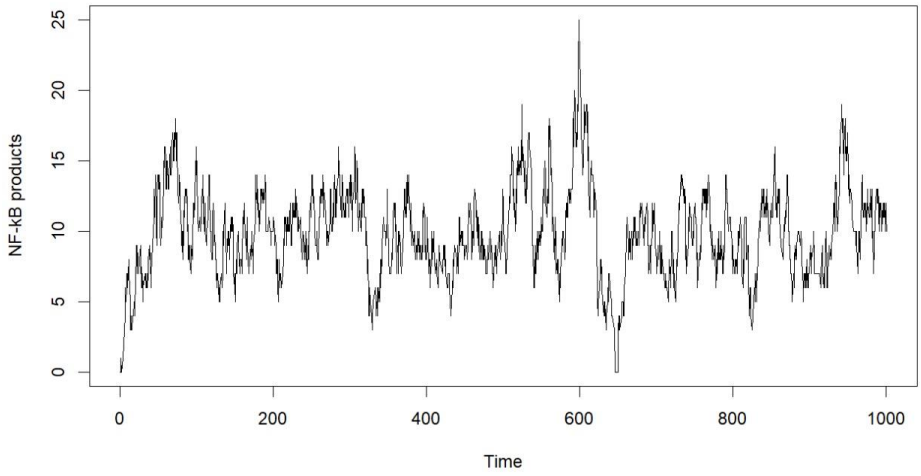

Fig 6: Oscillations in NF-kB products production

Discussion

In order to demonstrate the physiological importance of this model, a scenario of a cancer diagnostic center is taken. A single grid in a 96-well plate contains about 10 cells and let us say two reactions stimulating the NF-kB pathway are initiated in each of the 10 cells. As seen in Fig

7, 20 simulations with same initial conditions and rate constants were run to simulate the heterogeneity of cell behavior in context of immune or inflammatory responses.

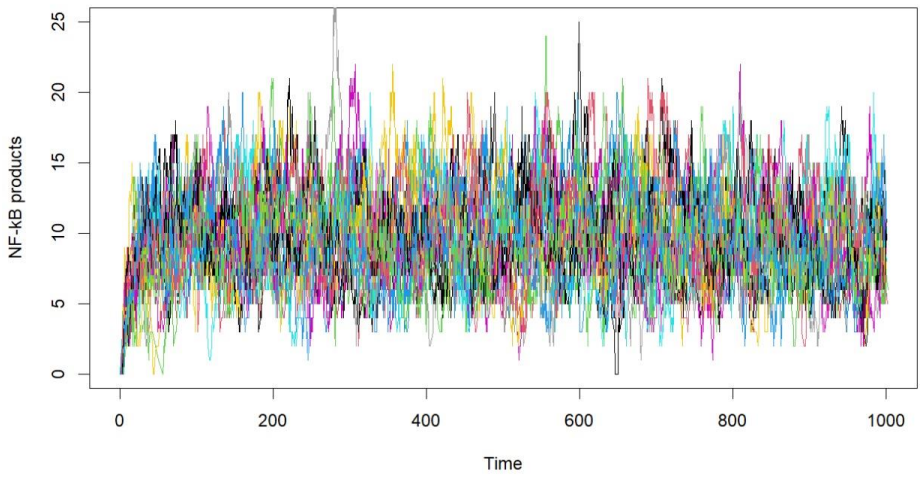

Fig 7: Stimulation of NF-kB pathway in 20 cells, 2 reactions per cell

The randomness of this pathway is reflected in the variability observed in Fig 7. Now, through Fig 8 let us look at the count and variability in total number of NF-kB products (mRNA transcripts) for the 20 different simulations we ran.

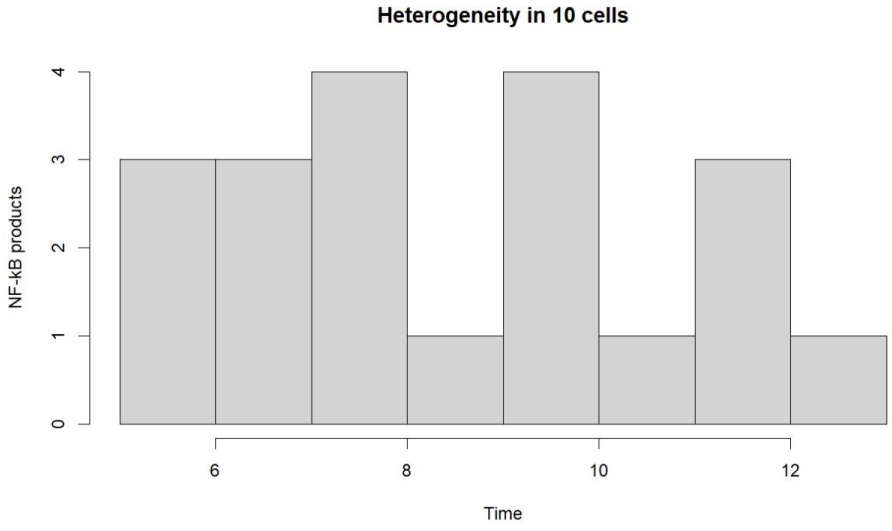

Fig 8: Histogram indicating the range of values for 20 simulations of NF-kB pathway

Conclusion

In conclusion, stochastic modelling of the NF-kB pathway is of utmost importance, especially in the field of cancer diagnosis where setting a threshold for target molecules/ the analyte decides the outcome of diagnosis. As discussed earlier, NF-kB pathway is misregulated in a variety of cancers as it can be taken advantage of by cancer cells to enable angiogenesis, avoid apoptosis and promote rapid cell proliferation. The model discussed in this report is a simple version that demonstrates the significance of incorporating stochasticity to the existing models for NF-kB pathway. This simple model can be built upon in order to be used for real use in projects pertaining to cancer diagnosis.

References

1. Model replicated from MD Thesis of Manuela Carriero: Modeling and data analysis of biochemical oscillators using Chemical Master Equation and AI: applications to the NF-kB activity in patient derived xenografts
2. Fundamentals of Systems Biology: From Synthetic Circuits to Whole-cell Models by Markus W. Covert
3. The IkappaB-NF-kappaB signaling module by Hoffmann et al., 2002