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Molecular and Stochastic Biophysical Modeling of mRNA Export and Quality Control

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Molecular and Stochastic Biophysical Modeling of mRNA Export and Quality Control

By

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Committee in charge:

Professor Mohammad R.K. Mofrad, Chair
Professor Shaofan Li
Professor Britt Glaunsinger

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Abstract

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Molecular systems orchestrating the biology of the cell typically involve a complex web of interactions among various components and span a vast range of spatial and temporal scales. Export and quality control of messenger ribonucleic acids (mRNAs) feature a prominent example of such an intricate molecular system. Export of mRNAs into the cytoplasm is a fundamental step in gene regulation processes, which is meticulously quality controlled by highly efficient mechanisms in eukaryotic cells. Despite extensive research on how mRNAs are quality controlled prior to export into the cytoplasm, the exact underlying mechanisms are still under debate. Specifically, it is unclear how aberrant mRNAs are recognized and retained inside the nucleus. Computational methods have advanced our understanding of the behavior of molecular systems by enabling us to test assumptions and hypotheses, explore the effect of different parameters on the outcome, and eventually guide experiments. In this dissertation, I present my research on mRNA quality control using different computational techniques.

Using the agent-based modeling (ABM) approach, which is an emerging molecular systems biology technique for exploring the dynamics of molecular systems/pathways in health and disease, we first developed a minimal model of the mRNA quality control (QC) mechanism. Our results suggested that regulation of the affinity of RNA-binding proteins (RBPs) to export receptors along with the weak interaction between the RBPs and nuclear basket proteins, namely myosin-like protein-1 (Mlp1) or translocated promoter region protein (Tpr), are the minimum requirements to distinguish and retain aberrant mRNAs. In addition, we demonstrated how the length of mRNA may affect the QC process.

The interaction between Mlp1 with one of the *Saccharomyces cerevisiae* RBPs, namely the nuclear polyadenylated RNA-binding protein (Nab2), was then investigated. Mlp1 plays a substantial role in mRNA quality control by interacting with other proteins involved in this process, specifically the RBPs. Yet, the mechanism of the interaction between Mlp1 and RBPs is still elusive. Using an array of integrated computational approaches including protein structure prediction, protein-protein docking, and molecular dynamics simulations, we dissected Mlp1-Nab2 interaction. Our results were consistent

with experimental observations, which suggested that Nab2 residue F73 is essential for Mlp1 binding and further uncovered an indirect role of Nab2-F73 in this interaction.

Dedicated to

My wonderful parents, Zahra and Akbar

For inspiring me to always be curious

My lovely wife, Mohaddeseh

For supporting and motivating me both personally and professionally to stay curious throughout this journey

And my beautiful son, Borhaan

For reminding me again how beautiful curiosity is.

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I would also like to thank my amazing parents and wonderful sister for their support and understanding. They have always supported me to reach my goals throughout my personal and academic life. Not being able to see each other for almost five years was a huge emotional burden, but they wholeheartedly supported me to follow my passion and dreams. I cannot thank them enough for all they have done for me.

And finally I want to thank my beloved wife and academic buddy, Mohaddeseh. She is not only a wonderful human being that has supported me in every aspect of my life, but also an achieved academic individual who has been a great help and support during my academic life. We have been through tough times together, but because of her patience and understanding, we always came out stronger. Our personal lives and mutual academic journeys started together, stayed to this day together, and will continue together forever.

Table of Contents

Chapter 1: Introduction

The work presented in this Chapter was adapted from the following published manuscripts:

M Soheilypour, M Mofrad, "Quality Control of mRNAs at the Entry of the Nuclear Pore: Cooperation in a Complex Molecular System", Nucleus. 2018

M Soheilypour, M. Peyro, Z. Jahed, M Mofrad, "On the nuclear pore complex and its roles in nucleo-cytoskeletal coupling and mechanobiology", Cellular and Molecular Bioengineering. 2016

M Soheilypour, M Mofrad, "Regulation of RNA-binding proteins affinity to export receptors enables the nuclear basket proteins to distinguish and retain aberrant mRNAs", Scientific Report. 2016

1.A: The Nuclear Pore Complex and Nucleocytoplasmic Transport

The nuclear envelope (NE) divides the interior of the eukaryotic cells into two physically separated regions, namely the cytoplasm and the nucleoplasm. Although a double-layered NE separates these two compartments, they are chemically and physically linked via two different protein complexes residing at the NE, namely the NPC (nuclear pore complex) and the LINC (linker of the nucleoskeleton and the cytoskeleton). The NPC acts as the exclusive gateway for macromolecular transport between the cytoplasm and the nucleoplasm, while the LINC complex is generally known as the physical linkage between the interior of the nucleus and the cytoskeleton.

The NPC is a large macromolecular complex composed of specific proteins called nucleoporins (Nups). More than 30 different types of Nups assemble into an eightfolded symmetric structure (Wolf & Mofrad 2008). The central channel of the NPC is sandwiched between the cytoplasmic and nuclear rings. Eight filaments project from the cytoplasmic ring into the cytoplasm and eight filaments emanate from the nuclear ring and form a basket-like structure, known as the nuclear basket (Jamali, Jamali, Mehrbod & M. R K Mofrad 2011) (Figure 1). The distinctive property of the NPC is the fast yet selective nature of transport it facilitates. Each NPC is capable of handling ~1000 translocations per second (Ribbeck & Görlich 2001). While the NPC allows free passage of small molecules and ions (up to 40 KDa), larger molecules (diameters of up to 40nm) require binding to specific types of proteins called transporters, e.g. karyopherins (Kaps), to be actively transported (Schmidt & Görlich 2016; Hoelz et al. 2011). Some of the Nups possess intrinsically disordered regions that line the inner face of the NPC (Denning et al. 2003). These Nups are rich in phenylalanine-glycine repeats, therefore called FG Nups, and are suggested to play the chief role in active transport of cargos through the NPC via formation of weak interactions with transporters. While various computational and experimental studies have explored the transport through the NPC (Rout 2003; Labokha et al. 2013; Mohaddeseh Peyro, Soheilypour, Ghavami & Mohammad R K Mofrad 2015; M Peyro et al. 2015; Yamada et al. 2010; Alber et al. 2007; Lim et al. 2007; Ando et al. 2013; Frey et al. 2006; Strawn et al. 2004; Koh & Blobel 2015; Moussavi-Baygi et al. 2011), the underlying mechanism of this process remains elusive.

1.A.1: Export and Quality Control of mRNA Transcripts from the Nucleus into the Cytoplasm

Messenger ribonucleic acids (mRNAs) are one of the major molecules that are transported through the NPC from the nucleus into the cytoplasm. Export of mRNAs is fundamental to various cellular functions in eukaryotes. Prior to export, the processing and packaging steps prepare a complex of mRNA and various proteins and protein complexes, collectively called messenger ribonucleoprotein (mRNP), enabled to exit the nucleus through the NPC and engage in production of proteins in the cytoplasm (Figure 2).

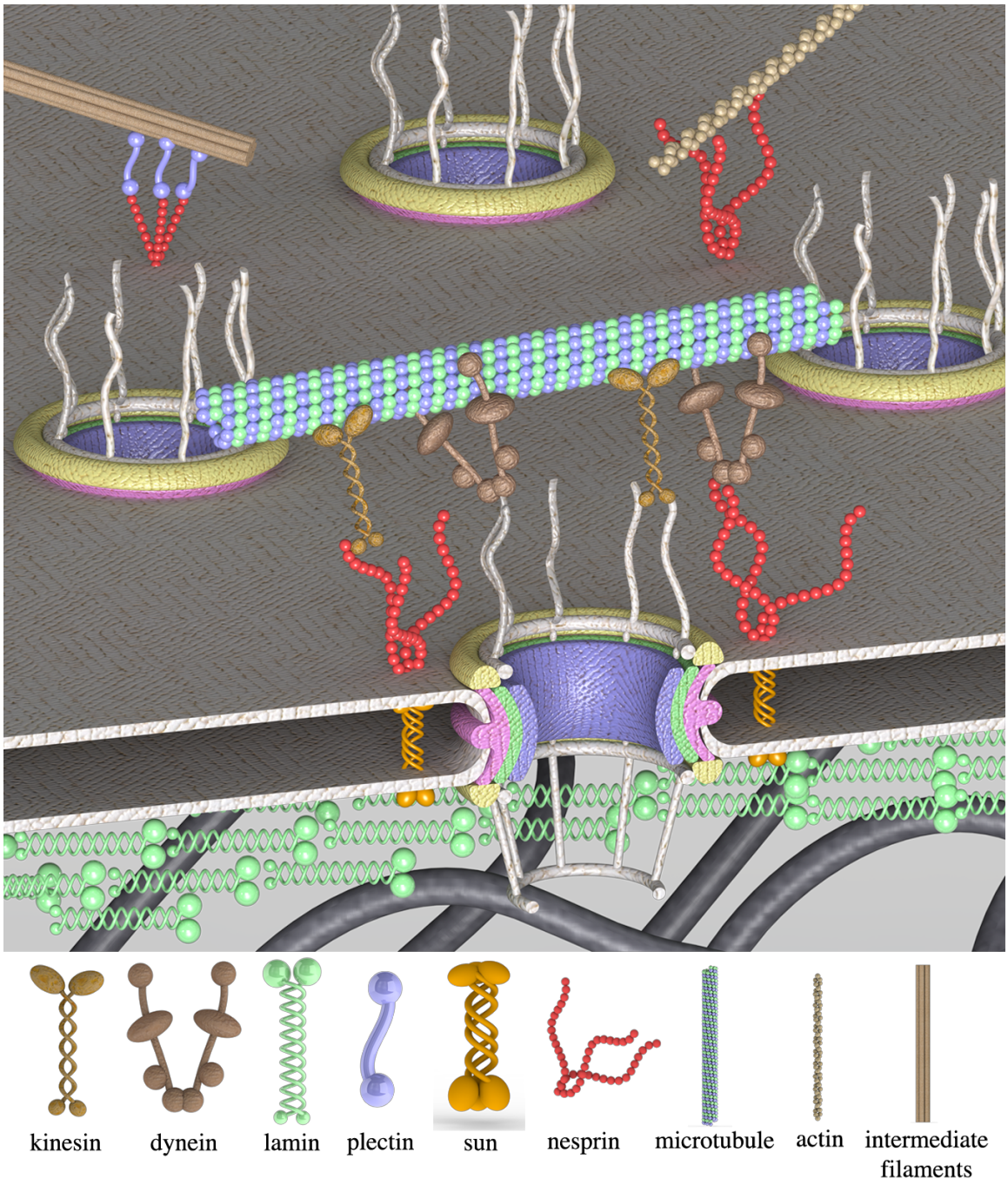

Figure 1: A 3-D schematic representation of the nuclear pore complex (NPC) embedded in the nuclear envelope (NE), as the sole gateway of nucleocytoplasmic transport, along with its interactions with the nuckleoskeleton and the cytoskeleton. The scaffold of the central channel of the NPC is made up of three layers of nucleoporins (Nups) shown in magenta, green, and pink (disordered regions of the FG Nups that fill the inner part of the NPC are not shown). Cytoplasmic filaments of NPCs associate with microtubule filaments in the cytoplasm through kinesin and dynein motor proteins (shown on the front NPC). Similarly, linker of the nuckeloskeleton and the cytoskeleton (LINC) complexes interact with cytoskeletal filaments, including microtubules (shown on front LINCs), actins, and intermediate filaments (shown on LINC complexes in the back that are not observable themselves), through nesprins and plectins.. Moreover, in the nucleoplasm, NPCs interact with nuclear lamina and chromatin.

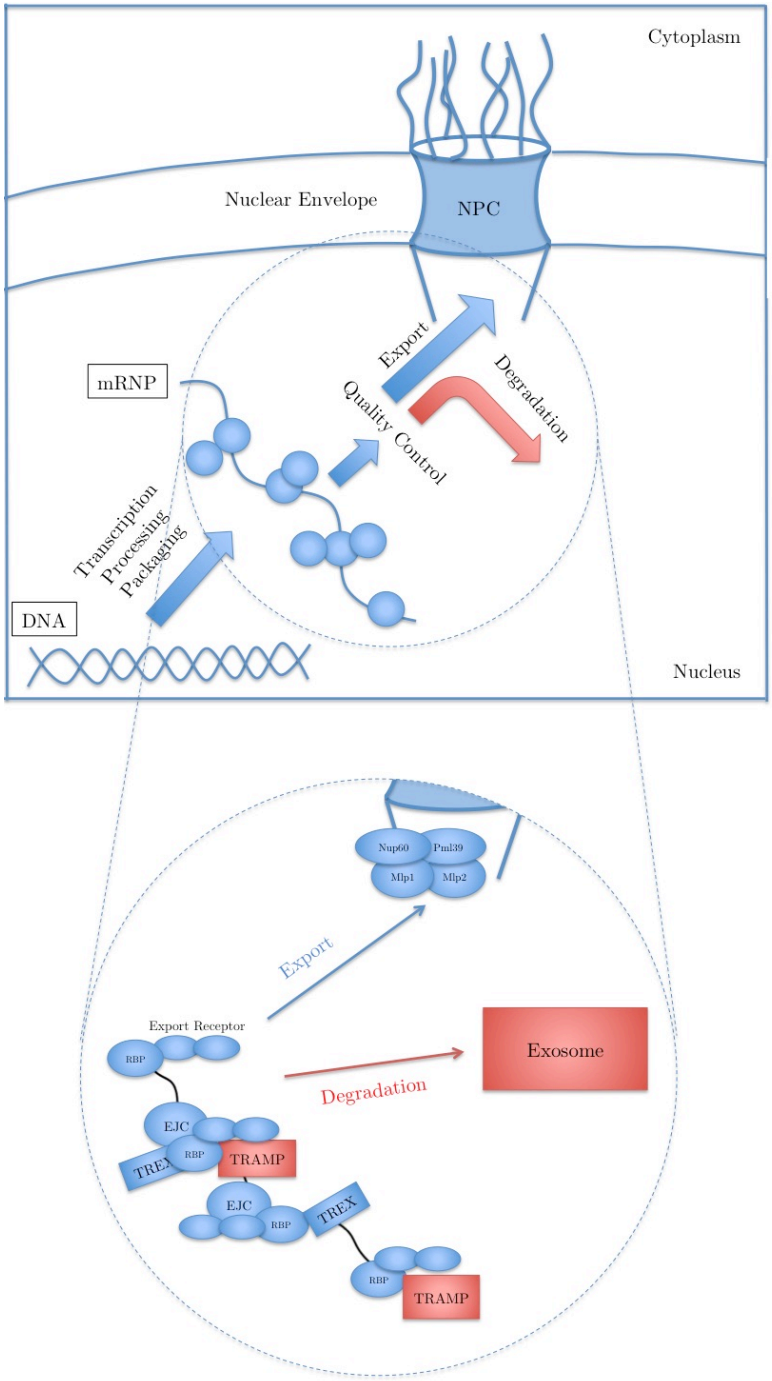

Figure 2: mRNA biogenesis in eukaryotic cells. Upon transcription, mRNA undergoes some processing and packaging steps, leading to the formation of messenger ribonucleoprotein (mRNP). Prior to export, mRNPs are quality controlled and either exported through the nuclear pore complex (NPC) into the cytoplasm or retained and degraded inside the nucleus. Successfully exported mRNPs engage in the translation process to produce proteins. Insert: The two pathways of mRNA's fate after transcription inside the nucleus. The export pathway (blue) involves multiple proteins and complexes including Exon junction complex (EJC), RNA-binding proteins (RBPs) (e.g. Nab2, Npl3, Gbp2, and Hrb1 in yeast (Lei & Silver 2002; Iglesias et al. 2010; Hackmann et al. 2014) and 9G8, SRp20, and ASF/SF2 in vertebrates (Huang et al. 2003)), and the TREX complex. Once mRNA undergoes all the processing and packaging steps, export

receptor heterodimers are recruited to facilitate the export of the mRNP complex. On the other hand, in the case of aberrant mRNAs (red), e.g. unspliced transcripts, RBPs recruit the TRAMP complex, which facilitates the degradation of mRNA by the nuclear exosome. Nuclear pore complex (NPC) associated quality control proteins (primarily Mlp proteins) ensure that only normal mRNAs are passing through the NPC and aberrant mRNAs are retained inside the nucleus for subsequent degradation by the nuclear exosome.

The NPC is filled with a set of intrinsically disordered proteins called FG (phenylalanine-glycine) nucleoporins or FG Nups that form a barrier for transport of cargos. Nuclear transport is, therefore, limited to either small molecules that could freely diffuse through this barrier or macromolecules that are bound to transporters or karyopherins. Transporters interact with FG Nups via their hydrophobic patches and carry the cargo through the nuclear pore (Jamali, Jamali, Mehrbod & M. R. K. Mofrad 2011). In the case of mRNA export, transporters are called nuclear transport receptors (NTRs) or export receptors, which enable the mRNA to pass through the NPC. Upon transcription inside the nucleus and prior to being exported into the cytoplasm, mRNAs are quality controlled to ensure the production of appropriately functioning proteins in the cytoplasm (Figure 2) (Tutucci & Stutz 2011). However, the mechanisms by which aberrant mRNAs, e.g. unspliced, are recognized and retained inside the nucleus are poorly understood (Hackmann et al. 2014; Porrua & Libri 2013).

1.A.2: Computational and experimental approaches to study mRNA export and quality control

Although mRNA export and quality control are explored with a range of experimental techniques, several unknowns still exist that are not easily tractable via experiments. For instance, the required density of export receptors that mRNA needs for an efficient export as well as how export receptor coverage on mRNA transcript affects mRNA export are still unknown (Azimi et al. 2014). In addition, the rate-limiting step of mRNA export through the NPC is still a matter of debate, with some experiments suggesting the nuclear basket (Siebrasse et al. 2012; Grünwald & Robert H Singer 2010), while others identifying the central channel of the NPC (Ma et al. 2013) as the rate-limiting step. Similarly, many aspects of mRNA quality control are still unclear. Besides the fact that the exact underlying mechanism is still a matter of debate, the minimum required factors are also unknown. In addition, how mRNA length affects the quality control process is unclear.

Computational models enhance our understanding of biological systems by allowing us to explore hypotheses and evaluate the effect of different parameters on the system behavior. They could also lead to predictions that could explain an experimental observation or be further examined using *in vitro* or *in vivo* experiments. Accordingly, our group recently developed an agent-based model (ABM) to explore mRNA export and quality control (Figure 3) (Azimi et al. 2014; Soheilypour & Mofrad 2016). Our model provides an efficient platform to explore the effect of different parameters in mRNA export and quality control (Chapter 2). Besides coarse-grained modelling, higher-resolution techniques, such as all-atom molecular dynamics would provide further information about the underlying mechanism of the interactions between different components of the system at the atomic level (Chapter 3).

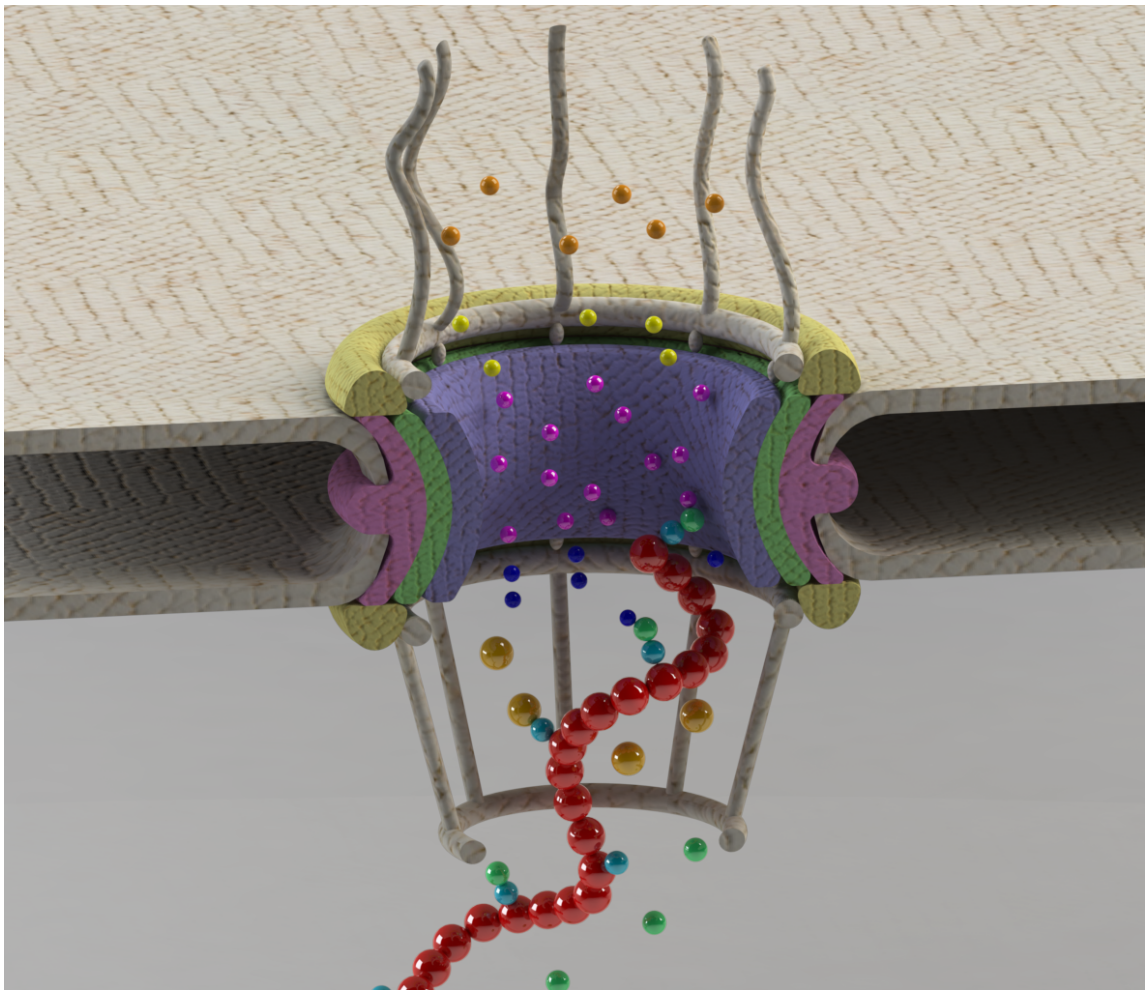

Figure 3. A schematic of agent-based modeling (ABM) of mRNA export and quality control. ABM is a bottom-up computational approach that simulates a complex system from the perspective of its constituents, molecules in this case. While proteins are modeled as single particle agents in corresponding regions of the environment, the mRNA sequence is modeled as a chain of monomeric agents, which can travel into different regions of the system.Agents move and interact (bind and unbind) with other agents according to a set of pre-defined rules associated with biophysical properties of represented molecules (Azimi et al. 2011; Azimi & Mofrad 2013). Coarse-grained representation of molecules enables ABM to easily achieve high temporal scales while maintaining a relatively high spatial resolution. Therefore, ABM is uniquely suited to explore different aspects of mRNA biogenesis. In addition, ABM can account for spatial details, which is the nuclear membrane as well as the specific donut-like shape of the nuclear pore complex (NPC). For the sake of visual clarity, a small fraction of actual concentrations is shown. Not drawn to scale.

1.A.3: Role of the Nuclear Pore Complex in Mechanobiology

Although NPCs are primarily known for their role as the gateway for 'chemical' exchange between the nucleoplasm and the cytoplasm, as discussed herein, they are also directly and indirectly involved in the 'physical' linkage between the cytoskeleton and the nucleoskeleton in various cellular processes. Since the biochemical aspect of NPC function has been widely discussed and reviewed previously, in this appendix, we focus specifically on the role of the NPC as a physical bridge between the nucleus and

cytoskeleton. First, the direct associations of NPCs with the nucleoskeleton and the cytoskeleton are discussed. Subsequently, we review the interactions between components of the NPC with the NE and how the NPC induces curvature into the NE and is assembled and integrated into it. The role of NPCs in actively transporting the inner nuclear membrane (INM) proteins, specifically components of the LINC complex, into the nucleus is then examined, which suggests an indirect role for the NPC in establishing the physical link between the nucleoskeleton and the cytoskeleton by regulating transport of INM proteins.

1.A.3.1: Associations of NPCs with the nucleoskeleton

Nuclear lamina is structurally coupled to the NPC

Nuclear lamina is a network of lamin filaments and lamin-binding proteins located near the inner nuclear membrane (INM) (Ho & Lammerding 2012). Lamins are type V intermediate filament proteins that can be categorized into two major types - A and B. Lamins are responsible for a wide variety of vital functions such as supporting the integrity of the nuclear membrane, NPC positioning, DNA replication, RNA transcription, nuclear and chromatin organization, cell cycle regulation and cell development, differentiation, migration, and apoptosis (Harada et al. 2014; Fiserova & Goldberg 2010; Gruenbaum et al. 2003; Hutchison 2002). The lamina network is suggested to act as a "molecular shock absorber" due to its extensibility and limited compressibility (Dahl et al. 2004). Nuclear lamina is structurally coupled to the NPC forming a network that inhibits independent movement of NPCs with respect to each other (Daigle 2001) (Chapter 1 : Figure 1). This connection is mediated by different types of nucleoporins. For example, Nup53 is tightly associated with the nuclear membrane and lamina and interacts with lamin B. Furthermore, it is suggested that Nup53 is positioned near the pore membrane and lamina where it anchors a subcomplex Nup93, Nup155 and Nup205 (Hawryluk-Gara et al. 2005). The NPC is also associated with the nuclear lamina through Nup153; depletion of Nup153 in Hela cells causes defective nuclear lamina organization. Nup153-depleted cells exhibit lobes and membrane invaginations in the nuclei.(Zhou & Panté 2010). Moreover, Nup153 has multiple binding sites for lamin types A and B, facilitating the interaction of both N-terminus and C-terminus of Nup153 with lamins. Mutations in lamin A (specifically in the Ig-fold domain) affect Nup153 binding, suggesting a role for Nup153 in lamin-associated diseases, i.e. laminopathies (Al-Haboubi et al. 2011). Lamins also prevent the aggregation of the NPCs on the NE in specific stages of the cell cycle (late G2 and prophase). NPCs are attached to dynein motor protein through Nup358 (discussed in section 2.1) and the movement of dynein on microtubules toward centrosome leads to clustering of the NPCs in the absence of lamins. NPCs are anchored to lamins, and lamins resist the force applied by dynein to the NPCs and therefore regulate positioning of the NPCs (Guo & Zheng 2015).

Chromatin

Nuclear periphery was previously thought to have no more than a repressive influence on gene expression. However, studies have shown that NPCs and genes are physically associated (Köhler & Hurt 2010). The physical interaction between the NPC and genes, which is mediated through physical adaptor proteins such as SAGA and TREX-2 (Köhler

& Hurt 2010) (for a comprehensive review, see (Köhler & Hurt 2010)) can regulate levels of transcription, increase the efficiency of mRNA processing and export, affect chromatin structure, transcription and interchromosomal clustering of genes within the nucleus (Schneider & Grosschedl 2007; Misteli 2007; Köhler & Hurt 2010). Moreover, some nuclear basket proteins such as Mlp1, Nup2 and Nup60 are involved in the interaction between genes and the NPC (Casolari et al. 2004; Schmid et al. 2006; Luthra et al. 2007).

Euchromatin is a lightly packed form of chromatin, which is often under active transcription. Heterochromatin, on the other hand, is a tightly packed form of chromatin, which is not transcribed. Heterochromatins are located adjacent to the NE and nuclear lamina, but are gapped with heterochromatin free zones that are associated with the NPCs. This association is mediated by nuclear basket protein Tpr. RNAi mediated depletion of Tpr leads to occurrence of heterochromatin all over the NE with even the NPCs being covered by heterochromatin (Krull et al. 2010). Hence, heterochromatin, which includes strongly repressed genes, localize near the nuclear lamina, while euchromatin localizes near the NPC (Belmont 1993; Schermelleh et al. 2008) (Kalverda et al. 2010). Genes in the euchromatin have different levels of expression and nuclear pore proteins interact with both highly and poorly expressed parts of the genome. Different categories of Nups correlate with different levels of gene expression (Sood & Brickner 2014). Some of the Nups have the ability to leave the nuclear pore and diffuse into the nucleoplasm for transcription (Hou & Corces 2010). Nups such as Nup60, Nup98, Nup50, Nup62 and Sec13 are found to bind specific regions of chromatin in the nuclear interior (Kalverda et al. 2010; Capelson et al. 2010). Studies have shown that in higher eukaryotes, nucleoporins inside the nucleoplasm mainly interact with highly transcribed genes. On the other hand, poorly expressed genes interact with NPC-tethered Nups (Liang et al. 2013; Kalverda et al. 2010; Sood & Brickner 2014). As an example, Nup98 is one of the Nups that can leave the NPC and has at least two pools: NPC-bound and nucleoplasmic (Oka et al. 2010; Griffis et al. 2002). Studies on Drosophila have shown that nucleoplasmic Nup98 localizes to promoters of genes that are substantial in processes such as development, and knockdown of this Nup results in suppression of these genes. These observations imply that Nup98 has a key role as a transcription factor in Drosophila (Franks & Hetzer 2013; Kalverda et al. 2010; Capelson et al. 2010). Therefore, cells might be able to regulate transcription in by controlling levels of nucleoplasmic Nup98 (Franks & Hetzer 2013).

LINC complexes

NPCs are additionally linked to the nucleoskeleton through close associations with elements of the LINC complex (Liu et al. 2007). LINC complexes are composed of inner and outer nuclear membrane proteins that interact in the perinuclear space (PNS). SUN (Sad1p/UNC (uncoordinated)-84) domain containing proteins, which are anchored to the inner nuclear membrane, interact with KASH (Klarsicht, ANC1 and Syne Homology) domain proteins, which are anchored to the outer nuclear membrane (Chapter 1 : Figure 1). Through the interactions of SUN proteins with lamins and chromatin, and the interaction of KASH domain proteins with microtubules, actin filaments and intermediate filaments, a direct physical linkage is formed between the nucleoskeleton and the

cytoskeleton (Roux et al. 2009; Horn et al. 2013; Liu et al. 2007; Sosa et al. 2013; Lombardi et al. 2011; Wilhelmsen 2005). Due to its interactions with Nup153, the LINC complex protein SUN1 is suggested to play a role in NPC-lamina interactions (Liu et al. 2007; Fiserova & Goldberg 2010; Li & Noegel 2015). Another nucleoporin, POM121 is also known to directly but transiently interact with LINC complex protein SUN1 to initiate NPC assembly (Talamas & Hetzer 2011).

1.A.3.2: Association of NPCs with the cytoskeleton

NPC components associate with microtubules and motor proteins at different stages of the cell cycle

Nups play different roles in association with various cytoskeletal elements, both in interphase and mitosis. The recruitment of Nup358, also known as RanBP2, to kinetochores during metaphase is essential for proper attachment of microtubules to kinetochores. Moreover, Nup358 is shown to interact with interphase microtubules through its N-terminal region (BPN) and regulate microtubule organization and cell migration (Joseph & Dasso 2008). Immunoprecipitation studies have shown that human Nup358 directly interacts with adenomatous polyposis coli (APC), which plays a role in microtubule reorganization, cell polarity, and migration (Murawala et al. 2009). In addition, with the aid of kinesin-2, Nup358 regulates the localization of APC to the cell cortex, a process that is independent of the nucleocytoplasmic transport. Therefore Nup358 is recognized as a localizer of kinesin-2 and APC to the microtubule ends in proximity of the cell cortex, which regulates the dynamics of microtubules and cell polarity (Murawala et al. 2009). Nup358 is also found to directly interact with BICD2 (mammalian homologue of the Drosophila Bicaudal D, which is an adapter protein between dynein motor and its cargos) in G2 phase of the cell cycle. BICD2, in turn, regulates centrosome and nuclear positioning prior to mitotic entry through regulation of dynenin and kinesin-1 (Splinter et al. 2010) (Chapter 1 : Figure 1).

Nups are also proposed to promote the attachment of microtubules and kinetochores as well as nucleation/stabilization of microtubules during mitosis.(Orjalo et al. 2006). For instance, Nup358, Mlp1, and Nup107-160 subcomplex relocalize to kinetochores (Orjalo et al. 2006; Salina et al. 2003; De Souza et al. 2009). Moreover, NPC is indirectly involved in mitosis checkpoints by recruiting Mad1/Mad2 to contribute to assembly of spindle checkpoint (Rodriguez-Bravo et al. 2014). Mad1 is anchored to the membrane by Mlp1/Tpr (De Souza et al. 2009) and is required for Mad2 localization (Rodriguez-Bravo et al. 2014). Nup153 is also suggested to be involved in localization of Mad1(Lussi et al. 2010). In the case of defective microtubule-kinetochore attachment, these two proteins (Mad1/Mad2) inhibit metaphase to anaphase transition (Gay & Foiani 2015).

These observations highlight the role of the NPC as a physical link to the cytoskeleton. These linkages could help regulate various cellular functionalities such as NPC distribution on the NE, as discussed earlier.

NPCs may indirectly associate with the actin cytoskeleton and intermediate filaments through their association with LINC complexes

To the best of our knowledge, there is no evidence on a direct interaction between NPC components and cytoplasmic actin filaments. However, a reorganization of the actin cytoskeleton upon depletion of Nup153 suggests some direct or indirect association of NPCs with actins (Chatel & Fahrenkrog 2012; Zhou & Panté 2010). A likely candidate for this association is through LINC complexes. As previously mentioned, components of LINC directly interact with actin filaments through KASH domain proteins (Chapter 1 : Figure 1). KASH domain proteins Nesprin 1 and 2 directly bind to actin via their calponin homology actin binding domains (Crisp et al. 2006; Sosa et al. 2012; Lombardi et al. 2011). Although completely speculative at this point, if SUN1 proteins can bind simultaneously with KASH proteins while associated with NUP153 (Liu et al. 2007), they can provide a direct link between the NPC and various elements of the cytoskeleton. As a result, NPCs may indirectly experience forces from actin and intermediate filaments, which may have implications in nucleocytoplasmic transport. Indeed, the SUN1-Nup153 interaction has shown to be an integral component of mammalian mRNA export. However, it remains unclear whether this interaction depends upon the direct linkage of SUN proteins with elements of the cytoskeleton

1.A.3.3: Interactions of NPCs with the NE

The process of NPC assembly into the NE is rather complicated, involving different types of Nups. Inducing curvature into the NE, directly interacting with the NE, or interacting with transmembrane proteins, different types of Nups cooperate to form the solid yet flexible structure of the NPC. Although there are some controversies in the field on specific details of this process as well as whether the structure of the NPC is rigid or flexible (Koh & Blobel 2015; Chug et al. 2015; Stuwe et al. 2015), the overall procedure is studied by various research groups. Here, we briefly outline some of the key observations about how NPCs assemble and integrate into the NE.

NPC assembly into the NE: post-mitotic versus interphase assembly

The central channel of the NPC is made up of three functionally distinct layers of Nups, namely the FG repeat layer, the scaffold layer (also called the adapter proteins layer), and the membrane layer (Lin et al. 2016; Devos et al. 2006; Jamali, Jamali, Mehrbod & M. R K Mofrad 2011) (Figure 1). NPCs assemble via different pathways at two different stages of the cell cycle. Some NPCs assemble at the end of mitosis, when the NE and NPCs assemble concomitantly, while the rest of the NPCs are assembled during interphase and integrated into the sealed NE (Doucet & Hetzer 2010). Several nucleoporins are identified as major regulators of NPC formation and assembly. In this section, we introduce the main role players, i.e. different Nups, involved in this process.

Independent of other proteins, Nup53 can directly interact with the nuclear membrane (Vollmer et al. 2012). It is shown that the RNA recognition motif domain, a substantial domain for functionality of Nup53 in NPC assembly, is necessary for the interaction of Nup53 with membranes. Nup53 has two membrane binding domains, one at the N-terminal domain and the other at the C-terminal domain, both of which require the middle

RNA recognition motif domain (Vollmer et al. 2012). The RNA recognition motif is primarily responsible for dimerization of Nup53 (Handa et al. 2006). Since this motif is required for Nup53 interaction with the NE, it is suggested that dimerization is also required for its interaction with membranes. Although either of the two binding sites is sufficient for NPC assembly at the end of mitosis, the membrane binding site at the C-terminus is specifically required for NPC assembly during interphase (Vollmer et al. 2012). On the other hand, in both yeast and metazoa, Nup53 interacts with transmembrane protein Ndc1, which is an essential interaction for NPC formation and assembly (Eisenhardt et al. 2014; Hawryluk-Gara et al. 2008; Mansfeld et al. 2006; Uetz et al. 2000; Onischenko et al. 2009); the N-terminal transmembrane domain of Ndc1 is necessary and sufficient for this interaction. The two observations, i.e. direct interaction of Nup53 with the NE versus the interaction of Nup53 with Ndc1, seem contradictory at the first glance. However, the interaction between Nup53 and Ndc1 is believed to counteract or fine-tune the membrane deformation capability of Nup53 for a proper NPC assembly (Eisenhardt et al. 2014). Alternatively, it is suggested that these two functionally redundant interactions offer different modes of association between the NPC and the membrane (Vollmer et al. 2012).

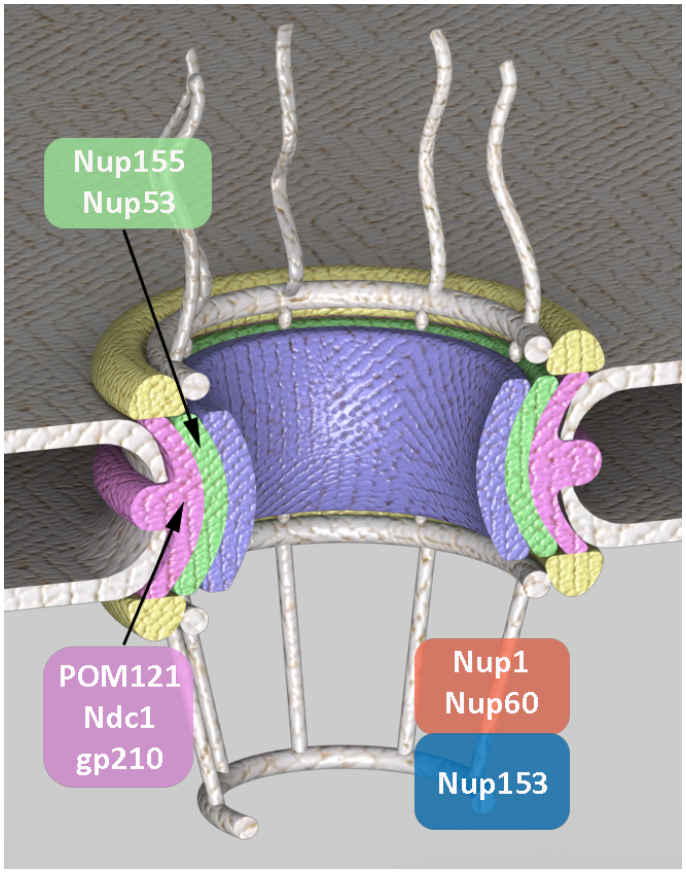

Figure 1: A closer view of the NPC scaffold. The central channel of the NPC is comprised of three layers of nucleoporins (Nups), namely the FG repeat layer (magenta), the scaffold layer (also called adapter proteins layer) (green), and the membrane layer (pink) (Devos et al. 2006; Lin et al. 2016). The FG layer is the innermost layer of the structure, where FG Nups, i.e. Nups rich in phenylalanine-glycine repeats, fill the inner part of the pore and facilitate transport of cargos. The scaffold layer is in between the other two layers

and involves Nups such as Nup53 and Nup155, which play substantial roles in NPC formation and assembly. The membrane layer is the third layer, which primarily consists of transmembrane proteins including POM121, Ndc1, and gp210. Some of the nuclear basket proteins, including Nup1 and Nup60 from yeast (labeled in the red box) and Nup153 from mammals (labeled in the blue box), are also suggested to be involved in NPC assembly into the NE. Only the Nups that are discussed in this review are shown.

Nuclear basket protein Nup153 is essential for interphasic NPC assembly but not for post-mitotic assembly (Figure 2). Through an amphipathic helix located at the N-terminus, Nup153 directly binds to the inner nuclear membrane, and, in turn, facilitates recruitment of the Nup107-160 complex (also known as the Y-complex), which is critical for NPC assembly (Vollmer et al. 2015). POM121, a transmembrane nucleoporin, is also found to be critical in integration of the Nup107/160 complex into assembly sites during interphase (Doucet et al. 2010). Conversely, ELYS is shown to be crucial for NPC assembly at the end of mitosis but not during interphase. ELYS is a nucleoporin that facilitates the recruitment of the Nup107/160 complex to chromatin (Doucet et al. 2010).

Nup155 is another nucleoporin that is suggested to be essential for membrane fusion and NPC assembly in both nematodes and vertebrates (Franz et al. 2005) (Figure 2). After the identification of early steps in NPC assembly that involve Nup107-160 complex as well as POM121 (Antonin et al. 2005; Harel et al. 2003; Walther et al. 2003), in vivo and in vitro studies demonstrated that Nup155 is subsequently recruited to these proteins and is required for NPC assembly (Franz et al. 2005).

Role of transmembrane proteins in NPC assembly

Nuclear pore proteins gp210 (Gerace et al. 1982; Wozniak 1989) and POM121 (Hallberg et al. 1993) have been identified to play a role in anchoring of the NPC to the membrane (Figure 2). Most of the structure of protein gp210 is localized inside the perinuclear space, while the small tail is exposed outside of the membrane (Wozniak 1989). Study of the exposed tail revealed that gp210 has an early role in NPC formation (Drummond & Wilson 2002). POM121 is also necessary for NE formation and NPC assembly in vitro (Antonin et al. 2005). On the other hand, while initial studies identified gp210 as an essential factor for NPC assembly, later studies argued any role for gp210 in this process. In line with this, while NPC assembly begins early in NE formation, gp210 is recruited to the membrane relatively late (Antonin et al. 2005).

Nucleoporins induce curvature into the NE

Recent in vitro and in vivo experiments reveled a direct interaction among nuclear basket proteins, namely Nup1 and Nup60, with the inner nuclear membrane (Mészáros et al. 2015) (Figure 2). Specific conserved domains of yeast Nup1 and Nup60 are shown to interact with nuclear membrane and induce membrane curvature. Amphipathic helices are commonly known to induce membrane curvature (McMahon & Gallop 2005). Bipartite motifs consisting of an amphipathic helix accompanied by an alpha-helical region are suggested to facilitate this curvature induction by combining both hydrophobic insertion and scaffolding mechanisms (Mészáros et al. 2015). This mechanism is supported by various observations including in vitro bending of membranes by Nup1 and Nup60 and in vivo reshaping of the nuclear membrane at high levels of Nup1. In addition, it was previously observed that Nup60 has some affinity to phospholipid

bilayers, lending more support to the role of Nup60 in inducing membrane curvature (Patel & Rexach 2008).

The putative viewpoint on the formation and assembly of the NPC depicts a rather complicated procedure involving several Nups and transmembrane proteins. One interesting aspect of such a complex structure could be associated with the hypothesis that NPCs could change their diameter based on cargo concentration inside the pore, i.e. demand for transport (Koh & Blobel 2015). However, this hypothesis has been debated (Stuwe et al. 2015; Chug et al. 2015). Ultimately, the association of all the involved proteins as well as protein complexes lead to formation of an octagonal structure with eight identical spokes. Theoretical studies have suggested that this eightfold symmetry maximizes the bending stiffness of each of the eight individual spokes, facilitating transport through the nuclear pore (Wolf & Mofrad 2008).

1.A.3.4: Indirect contribution of the NPC in establishing the linkage between the cytoskeleton and the nucleoskeleton

Proper localization of inner nuclear membrane proteins to the NE depends on their active transport through the NPC into the nucleus. Depletion of karyopherins importin-α and importing-β has been shown to disrupt the localization of Heh1 and Heh2 to the inner nuclear membrane (King et al. 2006). Lamin B receptor (LBR) is also found to bind Ran (Ma et al. 2007) and importins (Braunagel et al. 2007) and its translocation depends on the Ran function (Zuleger et al. 2011). Some of the LINC complex components also follow the same process. In C. elegans, localization of the SUN protein UNC-84 to the inner nuclear membrane is suggested to require active transport through the NPC to be able to appropriately localize to the inner nuclear membrane and form the LINC complex (Tapley et al. 2011). SUN2 is also found to possess a classical nuclear localization signal, as well as a perinuclear domain, that contribute to the localization of this protein to the inner nuclear membrane via transport through the NPC (Turgay et al. 2010). Therefore, these observations suggest an indirect role for NPC to regulate the formation of a physical bridge between the cytoskeleton and the nucleoskeleton in eukaryotic cells.

1.B: Quality Control of mRNAs at the Entry of the Nuclear Pore: Cooperation in a Complex Molecular System

Transport of messenger ribonucleic acids (mRNAs) from the nucleus into the cytoplasm is fundamental to various cellular functions in eukaryotes. Mutations or lacking of the components in mRNA export machinery have been linked to different human diseases (Lukong et al. 2008; Fasken & Corbett 2016). mRNAs are exported through the nuclear pore complexes (NPCs), the nanochannels that perforate the nuclear envelope (NE) and primarily act as a gateway for transport of various types of cargos (including mRNAs) into and out of the nucleus (see (Knockenhauer & Schwartz 2016; Jahed et al. 2016; Soheilypour et al. 2016; Raices & D'Angelo 2017; Dickmanns et al. 2015; Ptak et al. 2014) for recent reviews on different aspects of NPC structure and function). Upon transcription inside the nucleus and prior to being exported into the cytoplasm, mRNAs are quality controlled to ensure the production of appropriately functioning proteins in the cytoplasm (Chapter 1: Figure 2) (Tutucci & Stutz 2011). However, the mechanisms by

which aberrant mRNAs, e.g. unspliced, are recognized and retained inside the nucleus are poorly understood (Hackmann et al. 2014; Porrua & Libri 2013). Here, we present recent findings on mRNA quality control mechanisms, specifically at the entry of the nuclear pore complex (NPC), and the two hypotheses on the underlying dynamics of these processes. While one hypothesis highlights the "switch-like" behavior of the involved proteins as the key for mRNA quality control, an alternative hypothesis suggests that the efficient quality control is the emergent behavior of a combination of different regulated stochastic interactions between the involved components.

1.B.1: Export and quality control of mRNAs is a complex system involving a multitude of cooperating factors

To date, various methods and approaches have been employed to identify the underlying mechanisms of mRNA quality control. Using an array of techniques mostly involving knock out/knock down and/or mutation of target proteins, several proteins and protein complexes have been implicated in this process (for example see (Hackmann et al. 2014; Galy et al. 2004; Coyle et al. 2011; Palancade et al. 2005; Rajanala & Nandicoori 2012)). Some of these components are proteins/protein complexes that bind to mRNA, e.g. RBPs, as adapters that facilitate various stages of mRNA biogenesis. Other involved factors interact with these mRNA-bound components to fulfill these processes. While current research has identified various pieces of mRNA quality control machinery by identifying the different cellular components involved, details of the underlying mechanism are still unclear.

Although RBPs are considered as the main mediators in recruitment of export receptors to mRNA transcripts, this process may involve other participating factors. The exon junction complex (EJC), deposited 24 nucleotides upstream of exon-exon junctions upon splicing, is suggested to mediate the recruitment of export factor (NXF1) to mRNAs (Eberle & Visa 2014; Singh et al. 2012). However, analysis of human EJC and RNA interactomes reveals a physical association between EJC and SR proteins, which are RBPs featuring long repeats of serine and arginine amino acid residues. This observation might be a potential explanation for the functional overlap between EJC and RBPs (Singh et al. 2012). In addition, both Yra1 and its metazoan homologue Aly/REF interact directly with export receptors (Iglesias et al. 2010; Strässer & Hurt 2000; Stutz et al. 2000; Gatfield & Izaurralde 2002). However, Aly/REF is found not to be essential for mRNA export in Drosophila or Caenorhabditis elegans (Longman et al. 2003; Gatfield & Izaurralde 2002) and Yra1 is shown to be dispensable for mRNA export when an RBP (Nab2) and the export receptor (Mex67) in yeast are overexpressed. Therefore, Yra1 and Aly/REF are suggested to act more as cofactors for stabilization of the interaction between some of the RBPs and the export receptor (Iglesias et al. 2010). It is worth noting that, on the other hand, some studies have identified Aly/REF as a required factor for efficient mRNA export (Hautbergue et al. 2009; Okada et al. 2008). Interestingly, it is also suggested that some genes can tether to NPC components, which regulates mRNA expression (Ben-Yishay et al. 2016).

However, mRNAs do not directly recruit export receptors. Instead, RNA-binding proteins (RBPs) are key mediators that, on one end, bind to mRNA while, on the other end, recruit export receptors (namely, NXF1/NXT1 or Tap/p15 or Mex67/Mtr2), enabling the mRNA

to interact with FG Nups and pass through the NPC. To date, several different RBPs such as Npl3 (associates with mRNA close to the 5' cap) (Lei & Silver 2002), Nab2 (associates with mRNA at the 3' end) (Iglesias et al. 2010), Gbp2 and Hrb1 (associate with mRNA during splicing) (Hackmann et al. 2014) in yeast, and 9G8, SRp20, and ASF/SF2 in vertebrates (Huang et al. 2003), have been identified to facilitate acquisition of export receptors to mRNAs. Here, we have summarized the major factors involved in mRNA export, quality control, and nuclear degradation in Table 1.

In the next section, more details are provided regarding the role of each of these factors in their respected processes.

1.B.2: NPC proteins inhibit export of aberrant mRNAs

Under normal conditions, aberrant mRNAs that reach the NPC are not allowed to pass through, instead they are retained inside the nucleus and subsequently degraded. The NPC quality control step is achieved by a set of nuclear pore associated proteins including Mlp1, Mlp2, Pml39, and Nup60 (Coyle et al. 2011; Rajanala & Nandicoori 2012; Galy et al. 2004; Fasken et al. 2008; Hackmann et al. 2014; Palancade et al. 2005; Dziembowski et al. 2004). Among these, Mlp1 and Mlp2 (homologues of their human counterpart Tpr) are the most studied proteins and appear to be the main role players in NPC-associated quality control (Coyle et al. 2011; Rajanala & Nandicoori 2012; Hackmann et al. 2014; Fasken et al. 2008; Green et al. 2003; Bonnet & Palancade 2014). Pml39 and Nup60 are suggested to be upstream effectors for Mlp1 to localize it to the nucleoplasmic side of the NPC (Palancade et al. 2005; Fasken & Corbett 2009; Galy et al. 2004). While Mlp1 and Mlp2 are shown to associate with mRNPs (Niepel et al. 2013; Bretes et al. 2014; Green et al. 2003; Vinciguerra et al. 2005) they have no essential role in mRNA export (Strambio-de-Castillia et al. 1999; Kosova et al. 2000; Vinciguerra et al. 2005). However, overexpression of Mlp1 leads to mRNA accumulation in the nucleus and its deletion results in pre-mRNA leakage (Galy et al. 2004; Bonnet et al. 2015). Mlp2 is also suggested to function in quality control based on its enhanced interaction with mRNPs assembled in Yra1 mutant cells (Vinciguerra et al. 2005). The interaction of Mlp proteins with a multitude of mRNP components suggests that they function as a checkpoint for maturity of mRNPs prior to their export through the NPC (Hackmann et al. 2014; Fasken et al. 2008; Green et al. 2003; Niepel et al. 2013; Vinciguerra et al. 2005), allowing normally processed and packaged mRNAs to pass while retaining aberrant ones inside the nucleus. Interestingly, according to *in vivo* imaging studies, mRNAs spend 4-16 times more time at the nuclear basket compared with the central channel, which, besides mRNA remodeling at the nuclear basket, could be attributed to the quality control process (Grünwald & Robert H. Singer 2010; Grünwald et al. 2011). However, it is worth noting that it has been recently shown that under stress, heat-shock mRNAs bypass the NPC-associated quality control step and are rapidly exported (Zander et al. 2016).

Table 1 Proteins and protein complexes involved in mRNA export, quality control, and degradation. Yeast factors are presented with their metazoan counterparts in parentheses.

Protein or protein complex	Reference(s)
Mlp1 (Tpr)	(Coyle et al. 2011; Rajanala & Nandicoori 2012; Galy et al. 2004; Fasken et al. 2008; Hackmann et al. 2014)
Mlp2 (Tpr)	(Niepel et al. 2013; Bretes et al. 2014; Kosova et al. 2000)

Nab2 (ZC3H14)	(Anderson et al. 1993; Green et al. 2002; Hector et al. 2002; Schmid et al. 2015; Baejen et al. 2014)
Npl3	(Lei & Silver 2002; Shen et al. 2000; Baejen et al. 2014)
Gbp2 and Hrb1	(Hackmann et al. 2014; Tuck & Tollervey 2013; Baejen et al. 2014)
Pml39	(Palancade et al. 2005)
TRAMP complex (NEXT complex)	(Sakharkar et al. 2004; Porrua & Libri 2013; Hackmann et al. 2014; Lubas et al. 2011)
Nuclear exosome	(Chlebowski et al. 2013)
TREX complex	(Reed & Cheng 2005; Katahira 2012; Hackmann et al. 2014)
Yra1 (Aly/REF)	(Iglesias et al. 2010; Strässer & Hurt 2000; Stutz et al. 2000)
Mex67/Mtr2 (Tap/p15 – NXF1/NXT1)	
Exon junction complex (EJC)	(Eberle & Visa 2014; Singh et al. 2012)

Retained aberrant mRNAs are marked by the yeast Trf-Air-Mtr4 polyadenylation complex (TRAMP) for degradation (Sakharkar et al. 2004; Porrua & Libri 2013; Hackmann et al. 2014). Some SR proteins are suggested to facilitate this process by enabling a proper recruitment of the TRAMP complex or stabilizing its binding to aberrant mRNAs (Hackmann et al. 2014). The marked mRNAs will be subsequently degraded by the nuclear exosome, a multisubunit complex involved in processing and degradation of different types of RNAs (Chlebowski et al. 2013). Similar complexes and pathways are identified in human. The trimeric nuclear exosome targeting (NEXT) complex is required for exosomal degradation of promoter upstream transcripts (PROMPTs) (Lubas et al. 2011).

Upstream of these steps, transcription and mRNA export are tightly coupled via the evolutionary conserved transcription/export (TREX) complex (Reed & Cheng 2005; Katahira 2012). In yeast, this complex is composed of THO sub-complex (Hpr1, Tho2, Thp2, Mft1) and mRNA export adapter proteins (Sub2 and Yra1) (Reed & Cheng 2005). Similarly, human counterpart of the THO complex as well as Aly and UAP56 constitute the human TREX complex (Masuda et al. 2005). TREX-2 complex is recently shown to stably associate with the nuclear basket (Umlauf et al. 2013). However, despite the role of TREX complex in mRNA export, deletion of one of its elements (Mft1) as well as mutation of another element (Yra1) have no effect on the leakage of unspliced transcripts, implying that TREX has no direct role in the quality control of mRNAs (Hackmann et al. 2014).

Therefore, based on the findings to date, mRNAs are decorated with RBPs and once they undergo the required processing and packaging steps, RBPs recruit export receptors and facilitate the export of the resulting mRNP. On the other hand, Mlp proteins, at the nuclear basket, inhibit the export of aberrant mRNAs and RBPs that are bound to these mRNAs stabilize the binding of the TRAMP complex to facilitate their degradation (Chapter 1: Figure 2). As a simplified analogy, mRNA could be considered as an individual attempting to attend an event by purchasing tickets (RBPs), where multiple tickets are required for attendance. Tickets (RBPs) need to be certified by export receptors to be accepted. Finally, Mlp proteins represent guards at the entry that check the tickets and only allow individuals with a minimum number of certified tickets to pass. Nonetheless, how RBPs manage to determine mRNA's fate and what the distinctive

feature is that enables the cell to distinguish normal and aberrant mRNAs and retain the aberrant ones is under debate.

1.B.3: Cooperation in a complex molecular system: How aberrant mRNAs are recognized by NPC components and retained inside the nucleus

Various hypotheses are proposed regarding how mRNAs are quality controlled inside the nucleus (Wegener & Müller-McNicoll 2017). For example, an interesting mRNA biogenesis model suggests that mRNA quality control is a result of kinetic competition between mRNA processing and degradation, which is thoroughly discussed before (Porrua & Libri 2013). Here, however, we primarily discuss the hypotheses that consider the NPC components as essential parts of mRNA quality control. Quality control of mRNAs at the entry of the NPC is achieved by cooperation between several different sets of proteins and protein complexes and various research groups have sought to unveil how these different components cooperate with each other. Hackmann et al. recently identified two SR proteins in yeast, namely Gbp2 and Hrb1, and suggested that they function as switches that according to the state of mRNA, i.e. processed or not, recruit export receptors or the TRAMP complex for export or degradation, respectively (Hackmann et al. 2014). This mechanism identifies the SR proteins (which comprise most of the RBPs) as the key components to distinguish normal and aberrant mRNAs. This switch behavior is suggested to be achieved either according to the phosphorylation or methylation state of the SR proteins, or extended association of TRAMP. The latter suggests that mRNAs are initially associated with TRAMP and upon successful splicing, lose their association. Subsequently, SR proteins associate with export receptors, excluding their potential to bind to TRAMP (Hackmann et al. 2014) (Figure 1). Therefore, these SR proteins either bind to export-promoting factors or degradation-promoting components, therefore called switches (Hackmann et al. 2014) (we will call this mechanism as the switch mechanism).

Huang et al. previously studied two other SR proteins, namely 9G8 and ASF/SF2 in metazoans, and suggested that although the interaction of SR proteins with export receptors depends on whether mRNA is correctly processed or not, it only alters the affinity of the interaction, rather than completely eliminating the interaction; meaning that normal-mRNA-bound and aberrant-mRNA-bound SR proteins can both interact with export receptors. The results suggest that interactions between SR proteins and export receptors are modulated according to the state of mRNA, e.g. spliced or not (Huang et al. 2004). SR proteins are hyperphosphorylated when they co-transcriptionally bind to pre-mRNAs, and, upon splicing, become hypophosphorylated, i.e. partially dephosphorylated (Huang & Steitz 2005). Therefore, the phosphorylation state of SR proteins regulates their interactions with the target proteins. These SR proteins have been shown to be able to bind to export receptors when they are hyperphosphorylated (i.e. bound to pre-mRNAs), but with a lower affinity compared to when they are hypophosphorylated (Huang et al. 2004). Therefore, SR proteins that are bound to aberrant mRNAs, and are hyperphosphorylated, could still recruit export receptors rather than behaving as a deterministic switch according to the state of mRNA (we will call this mechanism modulated-affinities mechanism) (Figure 1). It is worth noting that the switch mechanism and the modulated-affinities mechanism are not mutually exclusive. The former suggests that SR proteins either bind to export receptors or the TRAMP complex; however, it does

not exclude the possibility of binding of aberrant-mRNA-bound SR proteins to export receptors with a lower affinity, which is suggested by the modulated-affinities mechanism.

Explaining the underlying mechanism of mRNA quality control using the switch mechanism is straightforward, where the ability to discern normal and aberrant mRNAs is attributed to the switch-like SR proteins, where aberrant mRNAs cannot recruit export receptors and, hence, are not able to interact with NPC proteins for export. However, it is not trivial to predict whether the modulated-affinities mechanism is sufficient for an efficient quality control of mRNAs, because in this hypothesis, aberrant mRNAs can still recruit export receptors and potentially get exported. From a complex systems standpoint, however, it is conceivable to hypothesize that the emergent behavior of the system, i.e. recognition and retention of aberrant mRNAs, is a result of the inter-molecular dynamics of the involved proteins with modulated affinities. This hypothesis, however, is not easily tractable using experimental approaches; partly due to the challenges in experimental studies that prevent researchers from exploring the *in vivo* dynamics of these processes and the factors involved with high spatiotemporal resolution (Heinrich et al. 2017). Therefore, we recently developed a computational model of mRNA export and quality control using a complex systems approach, called agent-based modeling (ABM) (Soheilypour & Mofrad 2016; Azimi et al. 2014). We sought to identify the 'minimal' factors required for mRNA quality control, since it is still unclear which factors are necessary for a successful quality control. Accordingly, we developed a minimal model for mRNA quality control composed of RBPs, export receptors, and NPC-associated quality control protein (Tpr or Mlp1). Using the model, we evaluated whether only regulating the interaction between RBPs and export receptors is sufficient for nuclear basket quality control proteins to distinguish normal and aberrant mRNAs. Our results showed that a lower affinity of aberrant-mRNA-bound RBPs to export receptors could enable Tpr/Mlp1 to distinctively retain aberrant mRNAs (by binding to individual RBPs), while allowing normal mRNAs to pass through the NPC, implying that even without switch-like behavior of some SR proteins, mRNAs could be discriminated in this minimal system. Retention of aberrant mRNAs at the nuclear basket provides extra time for nuclear machineries to degrade mRNA or perform processing steps, e.g. splicing (Bonnet & Palancade 2015). Our computational results imply that mRNA quality control does not necessarily require deterministic switches and, instead, the combination of regulated interactions could potentially discriminate normal and aberrant mRNAs (more on advantages of computational models in mRNA export and quality control in Box 1). It should be noted, however, that "active" involvement of the NPC and its constituents in mRNA export and quality control is still a matter of debate (please see further discussion in the "Conclusion and prospects" section).

The switch mechanism and the modulated-affinities mechanism share the same core idea, i.e. SR proteins bind to different factors depending on their modulation state. However, the two mechanisms lead to two different perspectives of the mRNA quality control mechanism, which influences future directions for experiments. The former suggests that we should identify proteins that act as switches, while the latter suggests that we should study the dynamics of the system as a whole with higher spatial and temporal resolutions. Conventional experiments only allow for bulk measurements. Therefore, in most of the

experiments, a potential component of the system is disturbed (e.g. knocked down) and the resulting effect, e.g. concentration of pre-mRNAs in the nucleus or the cytoplasm, is evaluated. These approaches only observe the system at discrete time intervals, ignoring the dynamics in between. However, recent advances in live cell single molecule imaging (SMI) (recently reviewed by Heinrich et al. (Heinrich et al. 2017)) could provide new tools for mRNA export and quality control studies and further clarify the details of these processes.

Presence of factors with redundant functions, such as EJCs and RBPs in recruiting export receptors (Singh et al. 2012), and some others as cofactors and stabilizers such as Yra1/Aly (Iglesias et al. 2010; Strässer & Hurt 2000; Stutz et al. 2000) reinforces the possibility of the modulated-affinities mechanism hypothesis by implying that extra regulatory considerations are required for a successful quality control. Nonetheless, it is also conceivable to suggest that both mechanisms are in place to provide a reliable, efficient quality control. The possibility of presence of yet-to-be-identified (switch-like) proteins that signal the quality control proteins to inhibit the export of aberrant mRNAs are also not excluded (Bonnet & Palancade 2015).

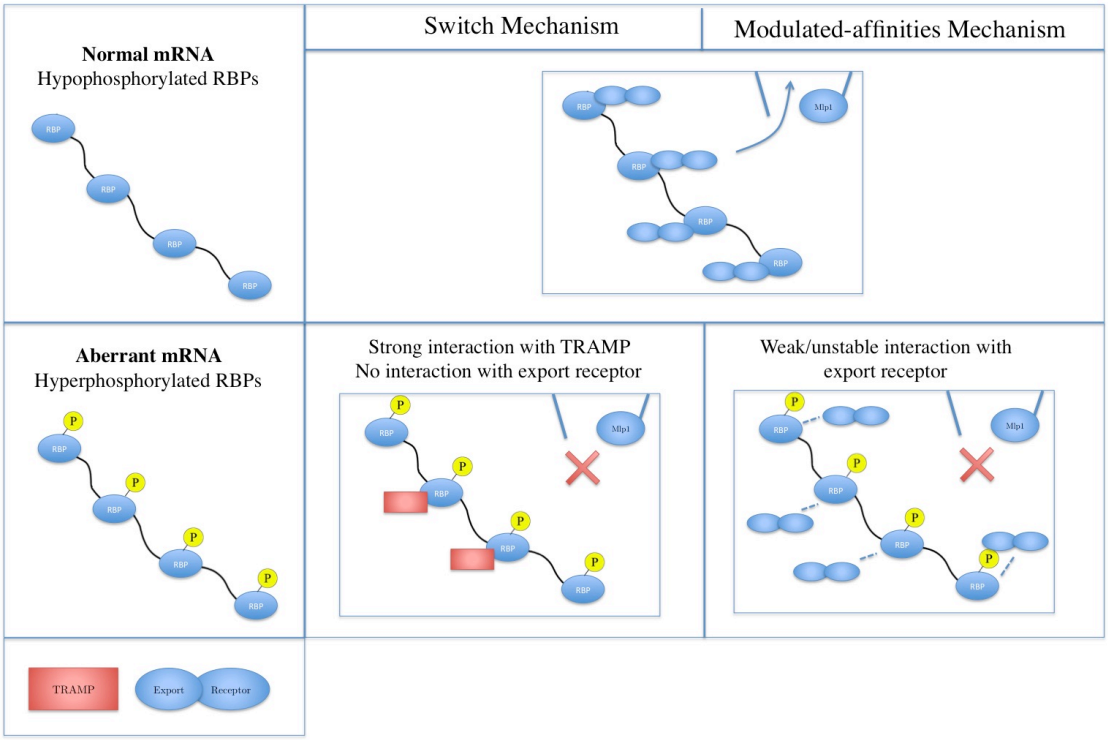

Figure 1: Comparison of mRNA quality control mechanism between the two hypotheses reviewed herein. The switch mechanism suggests that some RBPs, e.g. Gbp2 and Hrb1, do not interact with export receptors when bound to aberrant mRNAs, which is potentially achieved according to phosphorylation or methylation state of RBPs. Instead, these RBPs bind to the TRAMP complex for mRNA degradation. On the other hand, in the modulated-affinities mechanism, aberrant-mRNA-bound RBPs can still recruit export receptors, but with a low affinity. This hypothesis suggests that the weak/unstable interaction between hyperphosphorylated RBPs with export receptors is sufficient for the nuclear basket proteins, e.g. Mlp1, to distinguish aberrant mRNAs and retain them inside the nucleus (please refer to the text for more details).

One other aspect of mRNA quality control mechanism is whether it functions by selecting normal mRNAs (selection model), retaining aberrant mRNAs (retention model), or a combination of both (Bonnet & Palancade 2015). In the case of the selection model, Mex67 and Mlp1 are found in complex and are suggested to indirectly interact with each other (Niepel et al. 2013; Vinciguerra et al. 2005). The selection model is also supported by observations that suggest the nuclear basket as an interaction platform for passing mRNPs (Saroufim et al. 2015). Considering the wealth of information on the retention model, it is not conceivable to suggest that selection model is the sole mechanism for mRNA quality control. However, it could be the case that "retention" is the primary mechanism of quality control and "selection" further facilitates the process by providing a docking site for normal mRNAs to pass through the NPC more efficiently (Green et al. 2003). In line with the docking behavior hypothesis, it has been recently shown that SUN1, one of the components of the LINC complex (linker of the nucleus and the cytoplasm), has a significant role in mRNA export by interacting with the export receptors bound to mRNAs and eventually handing the mRNP to nuclear basket proteins for export (Li & Noegel 2015; Jahed et al. 2016).

Despite extensive research in nucleocytoplasmic transport and, specifically, mRNA export and quality control, the underlying mechanisms are still elusive. Computational methods could play a significant role in dissecting these intricate biological processes. In the following Chapters of this dissertation, I employ two computational approaches, namely agent-based modelling (Chapter 2 and Chapter 3) and molecular dynamics (Chapter 4), to explore mRNA export and quality control processes at different spatiotemporal scales and present how these computational techniques could provide valuable insights into these vital cellular processes.

Chapter 2:

Agent-based Modeling in Molecular Systems Biology

The work presented in this Chapter was adapted from the following published manuscripts:

M Soheilypour, M Mofrad, "Agent-based Modeling in Molecular Systems Biology", BioEssays. 2018

The computational trade-off of modeling molecular systems: "spatial resolution" versus "time scale"

The main challenge in molecular modeling is the complex relationship between spatial resolution and time scale, where increasing one would limit the other. The ideal scenario is to be able to model molecular systems with high spatial resolutions, i.e. nanometer, and for long time scales, i.e. seconds to minutes. This is currently not feasible due to the high computational expense. As a result, two categories of modeling techniques are employed, i.e. macroscopic and microscopic, each focusing on one of the two desired goals: high spatial resolution or extended time scales.

A well-established macroscopic method for modeling cellular pathways is bulk property models such as ordinary differential equations (ODE) of reaction rates that quantify concentration changes over time.(Savageau 1969; Gilbert et al. 2006) ODE representation of molecular reaction networks makes the assumption that (i) concentrations are high and (ii) the system is well mixed. (Szallasi et al. 2006) In some systems, the correlation length, or the length at which spatial homogeneity of reactants can be assumed, may be small—for example, reactions occur faster than the product species can diffuse to satisfy the well-mixed assumption. In such cases, spatial details should be considered through the use of partial differential equation (PDE) models. Both ODE and PDE models are well suited for systems with high concentrations that uphold the continuum hypothesis. However, the molecular systems often contain a discrete number of particles, which, in the time scales involved, fluctuates widely with respect to the characteristic length scale and the continuum hypothesis is not valid.(Szallasi et al. 2006; Kaznessis 2007) As a result, deterministic models such as ODEs and PDEs are not well suited for such systems.

Different models are developed to address problems involving a discrete number of particles such as the chemical master equation (CME), and the reaction diffusion master equation (RDME) (also see the Gillespie algorithm or the stochastic simulation algorithm (SSA),(Gillespie 1977) next reaction method,(Gibson & Bruck 2000) and reaction-diffusion SSA).(Bernstein 2005) CME and RDME are sets of deterministic ODEs describing the time evolution of a molecular system that is well-mixed or locally well-mixed (dividing the domain into sub-volumes and assuming each to be well-mixed). Although these models capture the stochastic nature of such systems, they apply the stochasticity to the population, not individuals.(Figueredo et al. 2014) Therefore, they cannot provide detailed spatial information about individual particles or individual particle tracking which is typically performed with much more computationally expensive Brownian dynamics (BD) or molecular dynamics (MD) techniques. On the other hand, BD and MD are not computationally able to handle large number of molecules involved in molecular pathways (Figure 1) (Lapin et al. 2010).

While macroscopic methods make simplifying assumptions to facilitate modeling of the target molecular system, microscopic methods, which are much finer in spatial resolution, are highly computationally expensive and cannot reach high temporal scales. This gap between the capabilities of computationally efficient macroscopic models such as ODE, PDE, CME and RDME and more detailed models such as BD and MD creates a need for mesoscopic modeling techniques, which can be satisfied using agent based models.

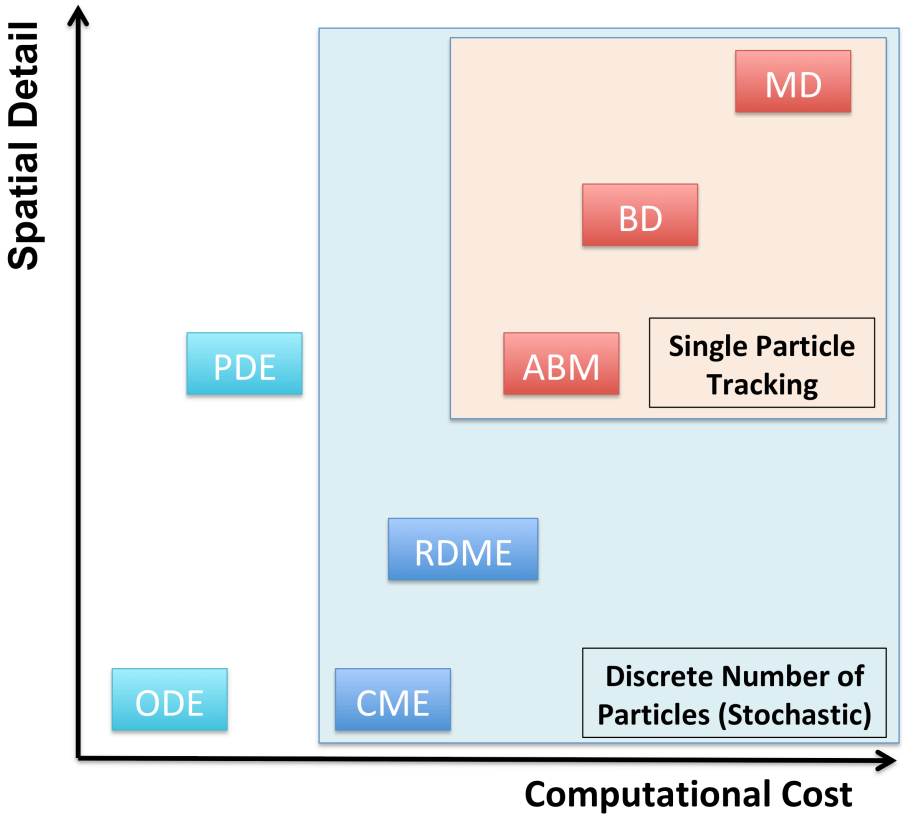

Figure 1. Spatial detail and associated relative computational cost for different computational modeling techniques for molecular systems. ODE: Ordinary Differential Equation; PDE: Partial Differential Equation; CME: Chemical Master Equation; RDME: Reaction Diffusion Master Equation; ABM: Agent-Based Model; BD: Brownian Dynamics (Gangel et al. 2013).

Agent-based modeling (ABM): Bridging the gap between high resolution and long time scale

Agent-based modeling (ABM) is a computational modeling paradigm that has been employed in a wide range of areas of research such as economics (Gangel et al. 2013; Dawid et al. 2014), social sciences (Helbing 2012; Conte & Paolucci 2014), environmental engineering (Xavier et al. 2007; Merkey et al. 2011), as well as biological studies including microbiome (Shashkova et al. 2016; Bauer et al. 2017; Hellweger et al. 2016), cancer (Zhang et al. 2009; Wang et al. 2015), and systems biology (Cannata et al. 2005; Montagna et al. 2008; An et al. 2009). ABM is a complex systems approach for simulating the interactions between multiple independent entities, termed 'agents', with the objective of assessing their individual effect on the overall system and predicting subsequent emergent phenomena.(Bonabeau 2002) Therefore, ABM is a bottom-up approach that models a complex system from the perspective of its constituent components.(Bonabeau 2002) Governing rules define how each individual agent moves and interacts, leading to reproduction of a complex phenomenon (Figure 2). ABM bridges the gap by observing the dynamics of the molecular system with a finer spatial

resolution compared to macroscopic methods, e.g. ODEs, while with a coarser temporal resolution compared to microscopic methods, e.g. MD (Figure 3).

Besides the discussed advantages of ABM over other computational techniques, several characteristics of ABM make it a perfect candidate for stochastic modeling of molecular systems. Agent-based simulations of molecular systems can achieve extended time scales. Many of the molecular pathways cannot be explored by high-resolution techniques such as BD because it is virtually impossible to reach meaningful time scales. For instance, export of mRNA transcripts through the nuclear pore complex (NPC) requires a millisecond time scale, which is beyond capabilities of MD or BD (Figure 3), but was recently explored through ABM (Azimi et al. 2014).

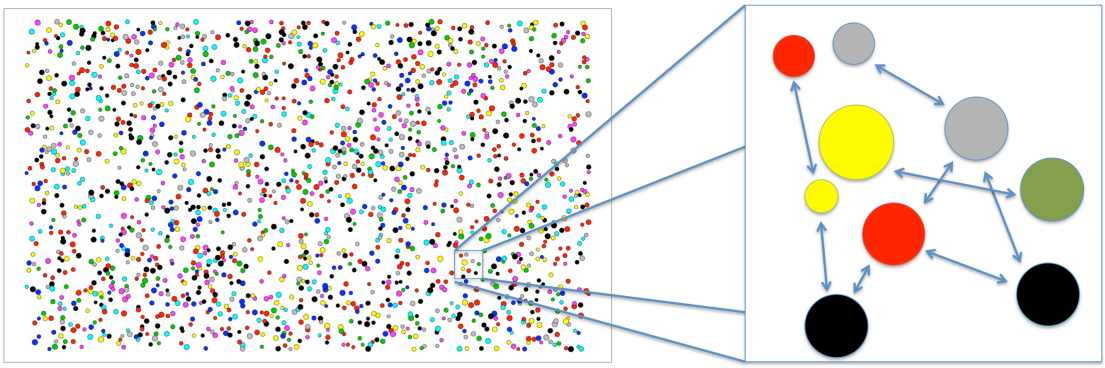

Figure 2. A schematic of agent-based modeling (ABM) of a complex system. The entities in the system, e.g. people, cells, or molecules, are simulated as "agents" (shown in different sizes and colors) that move and interact with each other. Each agent type has its own characteristics associated with their real-world properties. The complex web of interactions between the agents and the environment results in reproduction of a complex phenomenon.

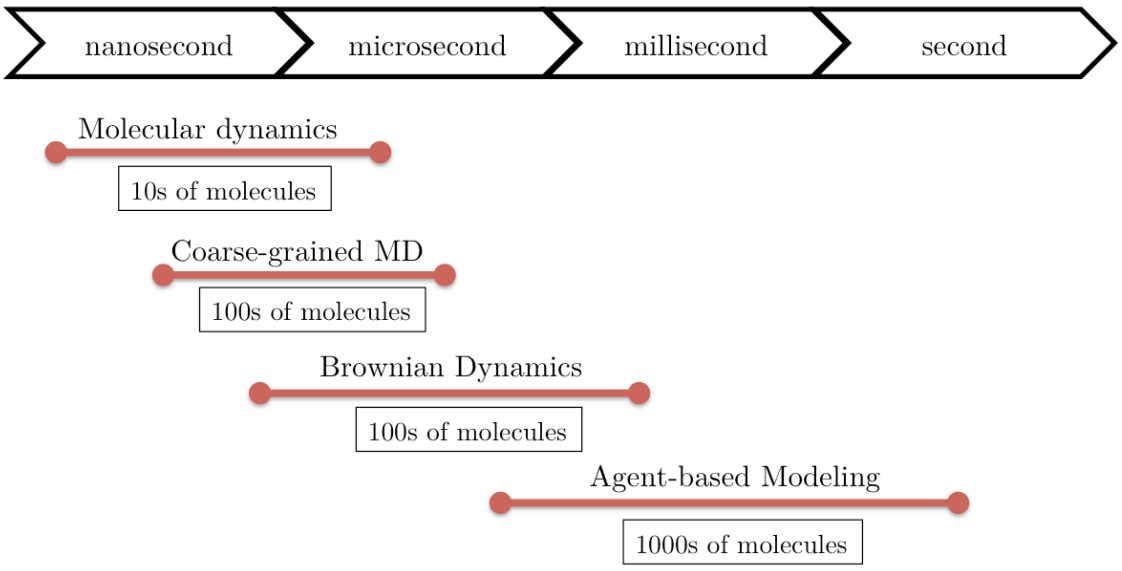

Figure 3. A comparison between the size of the system of interest and the timescales achievable by different molecular computational modeling techniques including molecular dynamics (MD),(Perilla et al. 2016; Truong et al. 2015; Shams et al. 2014) coarse-grained MD,(Mohaddeseh Peyro, Soheilypour, Ghavami & Mohammad R.K. Mofrad 2015; Ghavami et al. 2014; Grime et al. 2016) Brownian dynamics,(Moussavi-Baygi & Mofrad 2016; Ando & Skolnick 2014; ElSawy et al. 2013) agent-based modeling.(Soheilypour & Mofrad 2016; Azimi et al. 2014; Azimi & Mofrad 2013; Azimi et al. 2011; Jamali et al. 2013) Examples of studies employing these approaches are provided as references. ABM can easily achieve extended time scales for a relatively crowded system of molecules.

ABM also easily accounts for spatial details and constrained environments. Cells are composed of different compartments and most of the molecules are constrained to their associated environment. For example, while the linker of nucleoskeleton and cytoskeleton (LINC) is associated with the nuclear envelope (Jahed et al. 2014), RNA-binding proteins could travel between the nucleus and the cytoplasm, depending on their binding partners (Müller-McNicoll & Karla M. Neugebauer 2013). Representation of structural geometry and the local and non-homogeneous distribution of molecules, which is essential for many cellular processes, can be easily incorporated in ABMs.

Individual particles can be tracked in ABMs, which is also referred to as memory of past events (Figueredo et al. 2014). Study of molecular systems in the cell often requires a high resolution tracking of particles over the time of experiments or simulations. Accordingly, several efforts have been made to increase the spatiotemporal resolution of experimental approaches. For instance, in the case of mRNA export, while experimental approaches such as oligo(dT) in situ hybridization assay or single molecule fluorescence in situ hybridization (smFISH) can primarily perform bulk measurements to determine the intracellular distribution of RNA, they cannot capture high-resolution *in vivo* dynamics (Heinrich et al. 2017). Recent advancements in RNA labeling as well as imaging methods, however, have provided a platform to capture spatial and temporal dynamics of individual mRNAs *in vivo* (Grünwald & Robert H Singer 2010; Mor et al. 2010; Siebrasse et al. 2012; Smith et al. 2015). Similarly, in contrary to ODEs or even stochastic methods like Gillespie, individual particles could be tracked in ABMs over the course of simulation.

Moreover, ABM simulations can predict the emergent behavior of a complex system of molecules using the rules governing the behavior of individual molecules. The main objective in molecular systems biology is to understand the overall functionality of a molecular system and how different parameters affect this overall outcome. ABM, as a complex systems approach, has the ability to predict how a molecular system behaves given the rules that govern the behavior of individual molecules. Soheilypour and Mofrad,(Soheilypour & Mofrad 2016) for example, demonstrated how modulating the affinities between the components involved in mRNA quality control could substantially alter the outcome of the system, i.e. export versus retention of mRNAs.

ABM is able to efficiently capture the intrinsic complexity of biological pathways and unveil the influence of noise or disruptions of a single factor on the behavior of the system, which could be employed to explore the dynamics of disrupted molecular pathways in diseases. Considering these characteristics as well as incorporation of stochasticity to individual elements in the system (An et al. 2009; Figueredo et al. 2014),

which is an essential component in a molecular system, ABMs can be employed in a wide range of applications in molecular systems biology (Figure 4).

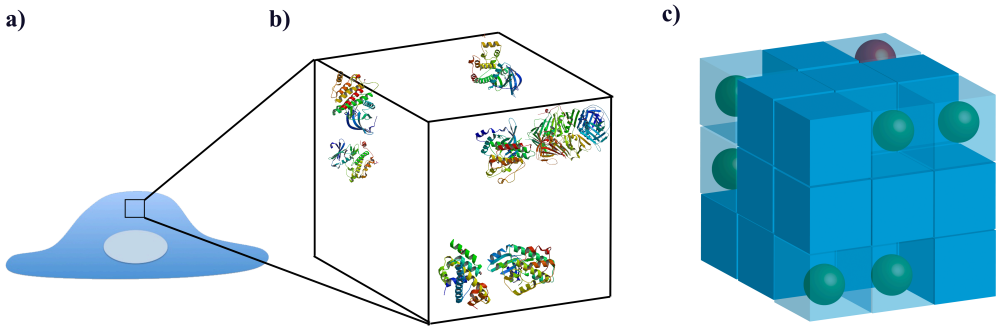

Figure 4. (a) Function of a cell is substantially dependent on various complex molecular pathways inside the nucleus as well as the cytoplasm. Each pathway takes place in a particular environment and involves several different factors, i.e. molecules. The cross-interactions between these molecules lead to the complex behavior of the system. (b) A zoomed-in view of seven proteins interacting in an imaginary molecular system in the cytoplasm. (c) Agent-based modeling (ABM) representation of the imaginary pathway shown in (b). Information about the environment is projected onto discrete cells. Agents representing biological factors move and interact with other agents and the environment based on the predefined governing rules. Agents interact with other agents only when they are in proximity of each other (green agents as oppose to the red agent).

Agent-based modeling tools

To date, several agent-based modeling and simulation tools are developed for different purposes. Table 1 summarizes some of the more popular general-purpose ABM tools (for a comprehensive review see Abar et al.) (Abar et al. 2017). Since ABM is a generic complex systems modeling approach, these ABM toolkits are designed to be applicable to a range of systems and problems in various fields of research, yet mostly tailored towards traditional ABM applications such as social behavior and macroscopic natural phenomena. However, they are not always considered the sole resource for ABM in some fields of research. For exmaple, although the available ABM tools are employed for complex biological systems at the cellular level such as epithelial renewal in skin (FLAME),(Li et al. 2013) retinal angiogenesis (NetLogo),(De Bernardi et al. 2017) tumor growth (MASON),(Ghadiri et al. 2017) bone remodeling(Paoletti et al. 2012) and Escherichia coli colony dynamics (RePast),(Tack et al. 2015) and pressure ulcer formation (SPARK),(Ziraldo et al. 2015) several ABM toolkits are developed specifically for cellular-scale studies including iDynoMics,(Lardon et al. 2011) BSim,(Gorochowski et al. 2012; Matyjaszkiewicz et al. 2017) BNSim,(Wei et al. 2013) and CellModeller(Rudge et al. 2012) and many other studies have developed their own in-house models.(Macklin et al. 2009; Poleszczuk et al. 2016; D'Antonio et al. 2012; Fortuna & Troisi 2010) The main reason, obviously, is that general-purpose ABMs are designed to fit a wide range of needs from significantly different fields of research. As a result, they lack specific features that one expects for a multicellular system. For instance, movement of microorganisms in aqueous environments is governed by Brownian

dynamics and flagellar forces.(Gorochowski et al. 2012) In addition, most of the cellular-scale ABMs use ordinary differential equations (ODEs) or partial differential equations (PDEs) to update molecular concentrations.(Kang et al. 2014) These features are lacking in general-purpose ABMs.

Table 1: Comparison of some of the general-purpose agent-based modeling tools. Most of these ABM tools are designed to fit a wide range of applications.

ABM Tool	License	Application Area(s)	Modeling Language	3D
FLAME (Kiran et al. 2010)	Academic license	General	XML+C	Yes
Mason (Luke et al. 2005)	Open Source	General (e.g. social complexity, swarm robotics, machine learning)	Java	Yes
NetLogo (Wilensky 1999)	Free	Social and natural sciences	NetLogo	Yes
Repast (North et al. 2013; North et al. 2005)	Open Source	Social sciences	Java	Yes
SeSAm (Klügl et al. 2006)	Open Source	General	Visual	Plug-in
Spark (Solovyev et al. 2010)	Open Source	Biomedical	Java	Yes
Swarm(Minar et al. 1996)	Open Source	General	Java	Yes

Similarly, molecular systems have specific features that differ from complex systems usually modeled via general-purpose ABM tools. While molecules have a mere reaction-diffusion behavior, agents in social sciences, e.g. humans, or microbial populations, i.e. microorganisms, are considered as intelligent and decision-making entities that demonstrate feedback or stimuli-based behavior. In addition, some molecules, such as DNA and RNA, are polymers, i.e. chain of monomeric agents, while there is no similar concept in larger-scale ABMs. Furthermore, diffusion and interactions of molecules are governed by well-established biophysical and biochemical rules instead of empirical observations. However, it is not technically impossible to implement such rules in general-purpose ABMs, as was done by Walpole et al.(De Bernardi et al. 2017) via NetLogo.(Wilensky 1999) In addition, most of the available ABM tools require a strong programming background for developing the models. This restriction limits the use of ABM to a relatively small group of users who are familiar with programming. As a result, most of the molecular systems biology studies have used in-house ABMs (Table 2).

Table 2: Examples of agent-based modeling studies of molecular systems. Because of the limitations associated with general-purpose ABMs, they are rarely employed in molecular systems biology.

Molecular System	ABM Tool	Spatial resolution / Size of the system	Time-step / Simulation Time	Number of Agents	Ref(s)
ErbB signaling	*in-house*	---	1 min / 100	Up to 1.5	(Das et al. 2017)

			min	million	
mRNA export and quality control	*in-house*	5 nm / $5.3x10^{-4}$ μm^3	2.5 μs / 20 s	1500-2000	(Azimi et al. 2014; Soheilypour & Mofrad 2016)
Integrin Clustering	*in-house*	0.01 μm / 1 μm^2	--- / 4 min	5000-10,000	(Jamali et al. 2013)
Toll-like receptor (TLR) 4 signaling	NetLogo	---	4 s / 1 min	A few thousands	(An 2009)
Intracellular signaling in prokaryotic cytoplasm	*in-house*	0.5 nm / 1000 nm^3	0.1 ns / up to 245 μs	Up to 1.7 million	(Ridgway et al. 2008)
NF-κB signalling pathway	*in-house*	27 μm^3	--- / up to 3500 s	<1000	(Pogson et al. 2006)

Bringing physical accuracy to computationally efficient ABMs

Agent-based models rely heavily on the rules governing the movement and interaction of agents. In a molecular system/pathway, the dynamics of molecules, movement and inter-molecular interactions, are governed by biophysical and biochemical rules. Therefore, one of the main challenges in using ABM for molecular systems is to directly relate the molecular properties, i.e. diffusion and interactions, to ABM parameters to ensure that the molecular ABM accurately represents the target molecular system/pathway. In an on-lattice ABM, where the modeling environment is discretized to a cubic lattice, it could be shown that according to the Fick's second law,(Fick 1995) the molecular diffusion could be related to the probability of movement as follows (Azimi et al. 2011; Ridgway et al. 2008):

$$D = P_{move}\frac{(\Delta L)^2}{\Delta t} \qquad \Delta t \to 0, \Delta L \to 0 \tag{1}$$

where D is diffusion coefficient, P_{move} is the movement probability, ΔL is the discretization length and Δt is the time step. Azimi et al. explored two potential diffusion mechanisms using this movement probability.(Azimi et al. 2011) An all-neighbor method, in which the agent searches for neighboring vacant grid cells results in an unnaturally higher effective diffusion coefficients. A single-neighbor method, however, was in agreement with Langevin Dynamics results as well as the analytical relationship. In a single-neighbor approach, the agent randomly picks a neighboring grid cell and if the cell is not vacant, the agent does not move at that time step.

First-order unimolecular reaction, i.e. molecular unbinding, and second-order molecular binding of two molecules could also be modeled using binding and unbinding probabilities. The reversible binding of two molecules A and B is given in Eq. (2).

$$A + B \rightleftharpoons AB\ (k_{on}\ and\ k_{off}) \quad (2)$$

It could be demonstrated that the following formula directly relate the binding and unbinding coefficients to ABM probabilities:(Azimi & Mofrad 2013; Ridgway et al. 2008)

$$P_{off} = k_{off}\Delta t \quad (3)$$

$$P_{on} = \frac{k_{on}\Delta t}{V/_{N_{Cells}} . N_{neighbors} . N_{Avogadro}} \quad (4)$$

where P_{off} is the unbinding probability, k_{off} is the unbinding coefficient, Δt is the time step, P_{on} is the probability of a binding between two neighboring molecules, k_{on} is the binding coefficient, V is the volume of the system, N_{Cells} is the number of grid cells, $N_{neighbors}$ is the number of von Neumann neighboring cells, e.g. 6 in a 3D lattice, and $N_{Avogadro}$ is the Avogadro's number. Using Eq. (4), Azimi and Mofrad(Azimi & Mofrad 2013) compared time-course data of a irreversible binding using a deterministic ordinary differential equation (ODE) versus the ABM probabilities, and demonstrated that ABM reproduces the average behavior of the ODE solution without the unnatural smoothness from a deterministic model.

Traditional ABMs mostly employ empirical sets of rules governing the behavior of agents in their environment. At the molecular scale, however, these algorithms do not necessarily represent the molecular species and their dynamics with enough accuracy. Therefore, the abovementioned direct transformations of biophysical and biochemical characteristics of molecules into ABM parameters, which are validated in several different studies,(Azimi et al. 2011; Azimi et al. 2014; Azimi & Mofrad 2013; Soheilypour & Mofrad 2016; Ridgway et al. 2008) provide a solid platform to more accurately model molecular systems and pave the path for wide utility of ABM in molecular systems biology.

Examples of molecular agent-based modeling

In order to demonstrate the wide utility and the potential of ABM in molecular systems biology, we summarize some of the studies that have taken advantage of ABM to explore the dynamics of molecular systems.

Diffusion behavior of molecules in the cell and subcellular compartments is critical in molecular pathways, specifically where molecular crowding and constrained environments limit the molecular interactions. Ridgway et al. created a virtual cytoplasm using an experimentally derived proteome of Escherichia Coli K12(Ridgway et al. 2008) and explored the effect of molecular crowding on *in vivo* cytoplasmic diffusion and diffusion-limited reactions. Similarly, Azimi et al. explored the effect of structural geometry on diffusion directionality in the cell cytoplasm using an in-house ABM(Azimi et al. 2011). More specifically, they explored how the structural geometry and orientation of actin filaments in the form of lamelliopodia versus filopodia affect the directionality of diffusion of actin monomers. They demonstrated that the parallel orientation of filopodia

and the quasi-random structure of lamellipodia give directionality to diffusion of monomers (towards the filopodia) at the filopodia-lamellipodia interface. In addition, it was shown that the angle between the filaments in the lamelliopodia is not directly related to the diffusion directionality and, instead, the web-like structure of the lamelliopodia hinders the diffusion of free actin monomers and results in the biased diffusion towards filopodia.

Different aspects of export of mRNA transcripts from the nucleus into the cytoplasm in eukaryotic cells are also explored via ABMs. Azimi et al. developed an agent-based model of mRNA export and explored a set of unanswered questions about this essential step in gene regulation processes.(Azimi et al. 2014) In order to be exported into the cytoplasm, mRNAs require a category of proteins called nuclear transport receptors (NTRs) that bind to mRNA and enable it to pass through the nuclear pore complex (NPC), i.e. the only gateway for transport of cargos between the nucleus and the cytoplasm.(Jamali, Jamali, Mehrbod & M. R. K. Mofrad 2011) The authors demonstrated that rate of mRNA export is dependent on the density and distribution of NTRs bound to the mRNA. In addition, previous experimental studies have reported contradictive results in terms of the rate-limiting step in mRNA export. While some studies identified that the rate-limiting step occurs at the nuclear basket of the NPC,(Grünwald & Robert H Singer 2010; Siebrasse et al. 2012) others have reported it to be at the central channel of the NPC.(Ma et al. 2013) The mRNA ABM was in agreement with the former observations, showing that the rate-limiting step was associated with reconfiguration of mRNA to thread itself into the central channel of the NPC. Furthermore, we recently explored the mRNA quality control mechanism.(Soheilypour & Mofrad 2016) Prior to export, mRNAs are quality controlled to ensure the production of appropriately functioning proteins in the cytoplasm.(Tutucci & Stutz 2011) Yet, how normal and aberrant mRNAs are distinguished and how the aberrant ones are retained inside the nucleus are still unknown.(Hackmann et al. 2014) Using ABM, we explored this process and demonstrated that regulation of the affinities between the involved components, i.e. RNA-binding proteins and NTRs, enables the nuclear basket proteins to distinguish normal and aberrant mRNAs, subsequently retaining aberrant mRNAs while allowing normal mRNAs to get exported (Figure 5). In addition, we examined how the length of mRNA affects the quality control process and predicted that retention of short mRNAs is more challenging. Since longer aberrant mRNAs spend more time in the nuclear basket to obtain a compact conformation for export, nuclear basket proteins have more time to capture and retain them inside the nucleus.

Different intracellular signaling pathways are also explored using agent-based models. An explored Toll-like receptor-4 (TLR-4) signal transduction pathway and the inflammatory response.(An 2009) Toll-like receptors, primarily located on inflammatory cells, are responsible for recognizing the bacterial cell wall products to initialize the body's response to infection.(Warren 2005; Zingarelli 2005) They demonstrated that agent-based representation of the TLR-4 signal transduction pathway could capture the stochastic signal behavior, dose dependent response, negative feedback control, and preconditioning effect. Pogson et al. also explored the NF-κB signaling pathway via ABM and showed that they could capture the dynamics observed by real-time single cell analysis.(Pogson et al. 2006) More recently, Das et al. studied the ErbB signaling

pathway (Das et al. 2017). ErbB receptors are responsible for propagation of signals throughout the cell to regulate cell proliferation, differentiation, migration, adhesion, apoptosis, and embryogenesis. As a result, overexpression of two receptors from the ErbB family, namely EGFR and HER2, has been attributed to different types of cancers.(Yarden & Sliwkowski 2001; Ahmad et al. 2011) The authors demonstrated that one could employ ABM to explore the different scenarios to re-engineer a signaling pathway in virtual experiments.

These examples demonstrate how ABM with its capabilities including particle tracking, accounting for stochasticity and spatial constraints, and the ability to predict the emergent behavior and reaching long time scales provides a platform to study complex molecular systems. It should be noted, however, that besides the advantages of ABM over other computational methods presented here, ABM has its own set of limitations as well. For instance, although an ABM allows modeling a molecular system with a higher spatial resolution over a longer time period, it also requires more details provided about the system of interest.(Pogson et al. 2006) For example, while ODEs only require the dissociation constant (Kd), ABM requires binding and unbinding coefficients (Kon and Koff) (please refer to equations 3 and 4), which are not always reported in experiments. Moreover, ABMs are significantly more computationally expensive compared to ODEs and PDEs. Nevertheless, most ABM simulations could be performed in a few hours and up to a few days on desktop workstations, depending on the size of the system.(Azimi et al. 2011; Rudge et al. 2012; Ghaffarizadeh et al. 2018) However, computational efficiency of ABM simulations heavily depends on their implementation. Efficient implementation of ABM simulations could result in linear (or close to linear) performance scalability.(Rudge et al. 2012; Ghaffarizadeh et al. 2018) In addition, for significantly large systems, ABMs could be easily parallelized with each computing node handling the calculations associated with a subset of agents. Accordingly, several efforts have been made to employ high performance computing (HPC) resources for large scale ABMs.(Dubitzky et al. 2012; Richmond et al. 2010; Rudge et al. 2012) ABM is also integrated with other methods, e.g. discrete-event simulation, to improve computational efficiency.(Montagna et al. 2016) It is also important to recognize that ABM, just like any other computational method, is only useful if employed in the right problem. For instance, in a well-mixed molecular system with high concentrations of the involved molecules, if the desired output is changes in concentrations over time, ABM and ODE would provide almost similar results,(Figueredo et al. 2014) while ABM simulations would be more computationally expensive.

Chapter 3:

Agent-based Modeling of mRNA Quality Control in Eukaryotic Cells

The work presented in this Chapter was adapted from the following published manuscript:

M Soheilypour, M Mofrad, "Regulation of RNA-binding proteins affinity to export receptors enables the nuclear basket proteins to distinguish and retain aberrant mRNAs", Scientific Report. 2016

Introduction

Following transcription, messenger ribonucleic acids (mRNAs) are transported to the cytoplasm to transfer genetic information and direct synthesis of functional proteins (Oeffinger & Zenklusen 2012). Multiple co-transcriptionally occurring processes applied on precursor mRNA (pre-mRNA) are followed by the engagement of several key proteins and complexes, including RNA-binding proteins (RBPs), along the length of pre-mRNA (Müller-McNicoll & Karla M. Neugebauer 2013; Tutucci & Stutz 2011), eventually forming an export-competent ribonucleoprotein (mRNP) prepared for efficient export through the nuclear pore complex (NPC). Splicing, 5′ capping, 3′ cleavage and polyadenylation are the four well-known processing steps prior to nuclear export, while failure in any of these steps yields aberrant mRNAs (Oeffinger & Zenklusen 2012; Tutucci & Stutz 2011). These processes are quality controlled by various evolutionary conserved and highly efficient mechanisms in eukaryotic cells (Tutucci & Stutz 2011; Eberle & Visa 2014). mRNA quality control (QC) occurs at different stages of RNA biogenesis (Porrua & Libri 2013) and is primarily dependent on intra-nuclear protein-protein interactions, wherein RNA-binding proteins (RBPs) play a major role (Katahira 2015; Dreyfuss, V Narry Kim, et al. 2002). Proteomic studies have suggested that lack of RBPs may result in different diseases such as cancer and neurodegenerative conditions (Lukong et al. 2008).

Several experimental studies have identified a multitude of proteins (such as nuclear basket associated protein Mlp1 (Tpr in human) and RBPs such as Nab2, Npl3, Gbp2, and Hrb1) that play a role in mRNA QC as their deletion or mutation in their genes lead to the leakage of pre-mRNAs into the cytoplasm (Fasken & Corbett 2009; Galy et al. 2004; Coyle et al. 2011; Rajanala & Nandicoori 2012; Palancade et al. 2005; Dziembowski et al. 2004; Lewis et al. 2007; Anderson et al. 1993; Green et al. 2002; Hector et al. 2002; Hackmann et al. 2014). Although most of these studies have been focused on yeast mRNA QC mechanism, similar proteins and complexes are identified in human (Lubas et al. 2011; Guo et al. 2003; Müller-McNicoll & Karla M Neugebauer 2013). However, despite the many studies conducted to understand the molecular events involved in mRNA QC processes, the exact mechanism through which mRNAs are recognized as incorrect remains elusive (Hackmann et al. 2014; Porrua & Libri 2013). More specifically, it is still unknown what are the minimum factors and interactions that are required to distinguish and retain aberrant mRNAs.

The major proteins involved in QC of mRNAs at the nuclear basket of the NPC can be categorized into two groups, 1) nuclear basket-associated proteins and 2) RBPs that are directly bound to mRNAs. The nuclear basket-associated proteins include Pml39, Tpr in vertebrates or Mlp1 and Mlp2 in yeast, and Nup60. Of these, Tpr/Mlp1 is suggested to play a key role in retention of aberrant mRNAs (Fasken et al. 2008; Green et al. 2003; Hackmann et al. 2014; Bonnet & Palancade 2014; Coyle et al. 2011; Rajanala & Nandicoori 2012). Mlp1 and Mlp2, homologs of Tpr in Saccharomyces cerevisiae, are not essential for cell growth and have been primarily suggested to possess a redundant function (Kölling et al. n.d.; Strambio-de-Castillia et al. 1999). Overexpression of Mlp1 is associated with nuclear accumulation of mRNA (Kosova et al. 2000) and its deletion leads to leakage of pre-mRNAs(Galy et al. 2004). Different research groups characterized

the interaction between Mlp1 and different RBPs and proposed Mlp1 as a checkpoint in the nuclear basket, which evaluates the maturity of passing mRNPs (Green et al. 2003; Fasken et al. 2008; Hackmann et al. 2014). Tpr is also suggested to possess the same role in mRNA QC (Rajanala & Nandicoori 2012; Coyle et al. 2011).

RBPs, as the second category of proteins involved in mRNA QC, are identified as recruiters of the export receptor heterodimer NXF1/NXT1 (Mex67/Mtr2 in yeast). Npl3 (Lei & Silver 2002), Nab2 (Iglesias et al. 2010), Gbp2 and Hrb1 (Hackmann et al. 2014) in yeast, and 9G8, SRp20, and ASF/SF2 in vertebrates (Huang et al. 2003) are the identified RBPs involved in this process. Other proteins, such as Yra1 and its human homologue Aly, are also suggested to regulate the recruitment of export receptors to mRNAs (Iglesias et al. 2010; Strässer & Hurt 2000; Stutz et al. 2000). However, they are found to be dispensable for mRNA export and act more as cofactors for stabilization of the interaction between RBPs and the export receptor (Iglesias et al. 2010).

Therefore, the current proposed model for mRNA export and QC at the nuclear basket (Iglesias et al. 2010; Hackmann et al. 2014; Tutucci & Stutz 2011) suggests RBPs as adapters for recruitment of export receptor heterodimers (presumably in concert with other elements as cofactors), while, upon initiation of mRNA export through the NPC, Mlp1/Tpr interacts with RBPs, acting as a checkpoint to verify the maturity of mRNP for export (Green et al. 2003; Fasken et al. 2008; Hackmann et al. 2014) (Figure 1). Nevertheless, it is still elusive how the collective behaviour of these factors and the kinetics of their interactions could efficiently distinguish aberrant and normal mRNAs.

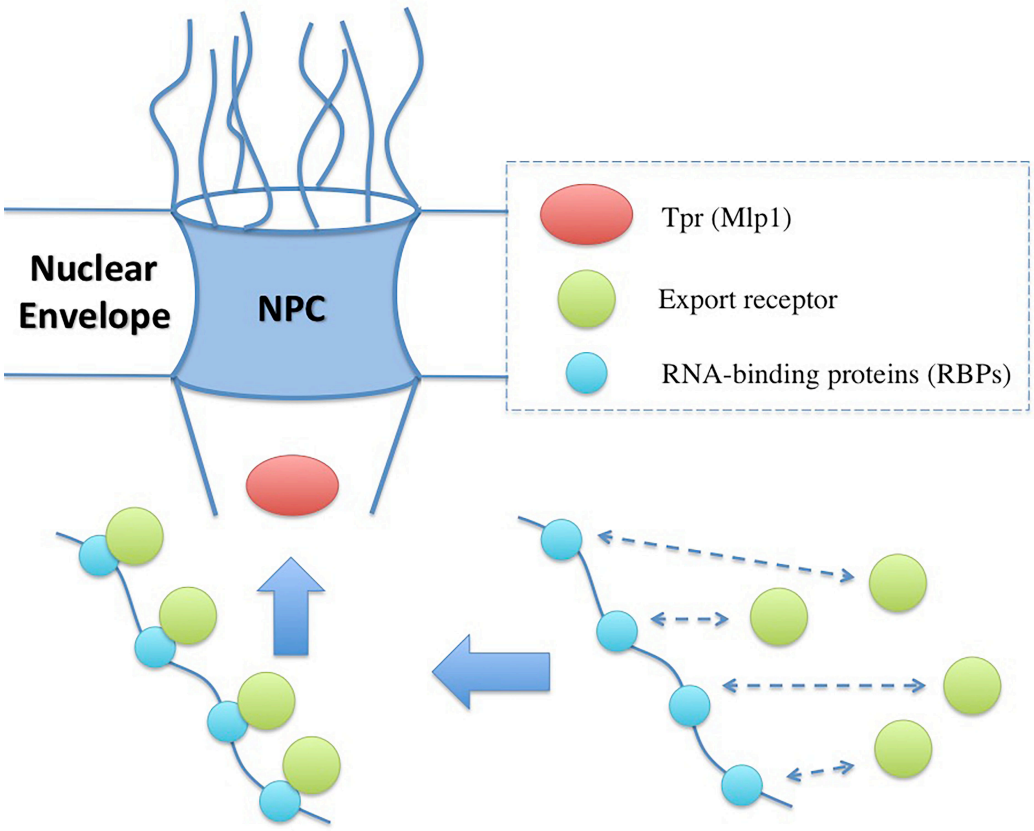

Figure 1: The currently proposed model for mRNA QC at the nuclear basket. RNA-binding proteins (RBPs), including Nab2, Npl3, Hrb1, Gbp2, 9G8, SRp20, and ASF/SF2, are suggested as adapters for recruitment of the export receptor heterodimer (NXF1/NXT1 or Mex67/Mtr2). On the other hand, nuclear basket protein Tpr (Mlp1 in yeast) interacts with RBPs and, as a checkpoint, verifies the maturity of the mRNPs. However, it is still unclear how the emergent behaviour of this system yields an efficient retention of aberrant mRNAs.

The lack of detailed understanding of mRNA QC mechanism could be partly attributed to the complex nature of this process, which makes it not easily tractable via experimental and conventional computational approaches. As a result, we employ a complex systems modelling approach, namely agent-based modelling (ABM), (Azimi et al. 2011; Azimi & Mofrad 2013; Azimi et al. 2014) to explore mRNA QC mechanism (Chapter 1: Figure 3). We seek to develop a minimal model for mRNA QC and identify the minimum required factors to efficiently distinguish and retain aberrant mRNAs. We hypothesize that the two abovementioned categories of the involved factors, namely RBPs and the nuclear basket associated protein Tpr (Mlp1 in yeast), are the major role-players in this process. Accordingly, we design a systematic procedure to demonstrate whether cross-interactions of these proteins could lead to proper retention of aberrant mRNAs. It should be noted that our model is, by no means, a comprehensive representation of the mRNA export and QC system, due to several unknowns that still exist (limitations of the model is thoroughly discussed in the Supplementary Information – section A). Instead, using the available experimental data, we seek to identify the minimal system that is able to distinguish and retain aberrant mRNAs. Our computational models, along with recent single-molecule imaging techniques (Smith et al. 2015; Bensidoun et al. 2016), could unveil valuable details of mRNA metabolism with a high spatiotemporal resolution.

Materials and Methods

Agent-based modelling (ABM): predictive modelling of complex biological systems

ABM is a promising approach to efficiently simulate the spatiotemporal interactions between multiple independent entities (agents) with the objective of assessing their individual effect on the overall system and confirming/predicting subsequent emergent phenomena. ABMs consist of a collection of agents with governing rules that dictate local behavior and interactions with adjacent agents, resulting in a complex emergent behavior that may not be obvious from the individual rules. Following the pre-defined environmental conditions, the agents move and locally interact with adjacent agents at each time step. Discretizing the space, on-lattice ABMs consists of a grid of "cells" which could be occupied by one or more agents. Each agent is only aware of agents within its neighbouring cells. The movement and interaction events, specifically defined for different types of agents, are based on some probabilities in conjunction with real-world governing rules for that specific agent. To date, only a few studies have taken advantage of on-lattice ABMs to study different biological systems (Bonchev et al. 2010; Devillers et al. 2010; Dong et al. 2010). Our ABM is specifically designed for modelling molecular diffusion, binding, and unbinding with consideration for physical factors such as molecular crowding and steric repulsion (Azimi & Mofrad 2013; Azimi et al. 2011; Azimi et al. 2014), and is capable of modelling complex three-dimensional biosystems in a computationally efficient and spatiotemporally detailed fashion. It is worth noting that

due to some limitations of mean-field approximation methods, it is inapplicable to use these methods to study mRNA export and QC mechanism (please see Supplementary Information – section C).

Transforming molecular movement and interactions into probabilities

We use the same method that we previously proposed for movement probability selection based on the following molecular diffusion probability, along with algorithms for realistic consideration of crowding and steric repulsion (Azimi et al. 2011; Jamali et al. 2013; Azimi & Mofrad 2013):

$$P_{move} = \frac{D.\Delta t}{\Delta L^2} \tag{1}$$

where movement probability of an agent is determined by its diffusion coefficient (D), simulation time step (Δt), and lattice discretization length (ΔL) (Ndimension, either 1, 2 or 3). Reduced probability method is implemented in the model to account for the steric effects of multiple agents occupying individual lattice sites (Azimi et al. 2011). Detailed derivation of movement probability from diffusion coefficient is explained in the Supplementary Information – section B. Probability selection of binding and unbinding events is based on our more recent work (Azimi & Mofrad 2013), where we proposed and validated the method to determine probability of binding and unbinding events from kinetic rate constants:

$$P_{on} = \frac{k_{on}\Delta t}{(V / N_{cells})N_{neighbors}N_A} \tag{2}$$

$$P_{off} = k_{off}\Delta t \tag{3}$$

The likelihoods are determined from real-world kinetic rate constants, i.e. koff and kon, simulation time step, Δt; system volume, V; number of lattice cells, Ncells; number of lattice neighbors that each cell has, Nneighbors; and Avogadro's number, NA. Detailed derivation of probability selection for binding and unbinding events is described in the Supplementary Information – section B.

ABM system and simulation details

Our ABM model is thoroughly verified and validated against other methods as well as *in vitro* and/or *in vivo* results and observations. The validation steps are comprehensively explained in the Supplementary Information – section D. In the model, mRNA is represented as a polymer of bound monomeric agents, which are limited in their movements through the constraint of maintaining connection with their nearest neighbors. These agents are only allowed to move in the diagonal direction into a nearest neighbor's von Neumann neighborhood to ensure that all movements are of the same length and all movement events of a specific agent type could be accommodated by a single movement probability. The model environment consists of a 42,108-element, three-dimensional lattice composed of cubic elements with dimensions of 5nm x 5nm x

5nm. The lattice size was selected to accommodate the volume associated with the Stokes radius of the largest single-agent species in the system, in this case a collection of nucleotides representing twice the persistence length of the mRNA, or Kuhn length (ranging from ~0.5 to 3 nm (Magee & Warwicker 2005; Vanzi et al. 2005)). Additionally, the model allowed for multiple agents of the same or different species type to occupy the same lattice element at any given time, so long as the available volume of a lattice element was not exceeded by agents diffusing into it. Discrete lattice elements belong to one of six region types: cytoplasmic, nuclear membrane, nucleoplasm, cytoplasmic filament periphery, central channel, or nuclear basket. The cytoplasmic region contains a high concentration of Dbp5 in complex with Gle1 and IP6 while the nucleoplasm in each simulation contained a single mRNA, discretized into a number of agents (Chapter 1: Figure 3). The 35nm-thick nuclear membrane which partitions the two compartments is impermeable to all agent types and contains a single nuclear pore with a diameter of 30nm at the center and 50nm at the peripheries. The cytoplasmic filament periphery consists of a 50nm diameter region that extends 30nm into the cytoplasm while the nuclear basket is composed of a basket shaped region that extends 55nm into the nucleoplasm (Adam 2001; Löschberger et al. 2012). Due to the eight-fold symmetry of the NPC (Szymborska et al. 2013; Löschberger et al. 2012), the cytoplasmic periphery, central channel, and nuclear basket each contain 24, 80, and 32 agents, respectively, representing the distribution of FG Nups (Yamada et al. 2010). In addition to these FG agents, non-FG agents are added to the channel to represent regions of the Nups that lack affinity for transport receptors but play a role in sterically repelling molecules, with the sum of the volume of these Nups corresponding to experimentally reported volumes (Yamada et al. 2010) (please refer to (Soheilypour et al. 2016; Jahed et al. 2016; Knockenhauer & Schwartz 2016) for recent comprehensive reviews on NPC and (M Peyro et al. 2015; Mohaddeseh Peyro, Soheilypour, Ghavami & Mohammad R.K. Mofrad 2015; Ghavami et al. 2014; Sakiyama et al. 2016; Ando et al. 2013) for detailed analysis of the behaviour of FG Nups). The collection of agents representing the mRNA is free to diffuse throughout the system while FG agents and non-FG agents are restricted to movement within their respective pore regions in order to maintain the permeability barrier. Chapter 1: Figure 3 schematically shows the model used in this study.

The nucleus contains a certain number of NXF1/NXT1 export receptors, which can bind to RBPs bound to the mRNA sequence. RBPs are evenly distributed along the mRNA sequence according to the desired density. The affinity between different RBPs and NXF1/NXT1 was assumed to be the same. This affinity was taken from a recent *in vitro* study, which was reported to be approximately 0.09μM (Teplova et al. 2011). Accuracy of the affinity to mimic mRNA export is evaluated (please see the Supplementary Information – section D). Population of NXF1/NXT1 inside the nucleus was varied across simulations. In order to represent aberrantly processed mRNAs, the affinity between RBPs and NXF1/NXT1 was decreased by 10 and/or 100-fold (please see the Results section for the reason for decreased affinities). These values are chosen to be much higher than the variations in the measured affinity for different lengths of mRNAs (Teplova et al. 2011). For each configuration, 100 replicate simulations were generated and analyzed. Where necessary, the 100-replicate simulations were repeated three times. Each simulation contained a single mRNA with a random initial configuration inside the

nucleoplasm. Each simulation was carried out for the duration of 20 seconds using a timestep of 2.5 μs. In our model, we have assumed that Tpr has the same interaction with all RBPs bound to the mRNA sequence. The affinity of the interaction, i.e. ~1μM, is taken from an *in vitro* study (Fasken et al. 2008). Moreover, Tpr only interacts with RBPs that are not bound to export receptor, because there is no experimental evidence on the interaction between the complex of RBP-NXF1/NXT1 and Tpr. This is further discussed in the Discussion section. The location of the 5' and 3' termini along with the number of mRNA monomers passed the NPC were tracked over the course of the simulation. The trajectories were analyzed to determine the fraction of successful transports per configuration. mRNAs that have completely passed the NPC were considered successful transport events. As we previously showed that a double-tag approach provides more realistic results compared to single-tag labelling (Azimi et al. 2014), here, a double-tag approach is adopted to track mRNAs.

Results

We seek to test our hypothesis that the interactions between three types of proteins involved in mRNA export and QC, i.e. the export receptor, RBPs, and Tpr, are the key to distinguish aberrant mRNAs. Accordingly, we take a step-wise approach: first, the interaction between Tpr and RBPs is inhibited in the model to evaluate whether only regulation of the interaction between RBPs and export receptors is sufficient to retain aberrant mRNAs or not. We evaluate this system under different environmental conditions for different configurations of mRNAs. Subsequently, in the next step, we implement the interaction between Tpr and RBPs.

Is regulation of the interaction between RBPs and the export receptor sufficient to retain aberrant mRNAs inside the nucleus?

Splicing is not an absolute requirement for the indirect interaction of export receptors with RNA (Reichert et al. 2002; Rodrigues et al. 2001), and the export receptor in metazoans (TAP) has a higher affinity to RBPs bound to spliced mRNAs, compared to RBPs bound to unspliced mRNAs (Huang et al. 2004). Therefore, RBPs bound to aberrant mRNAs could still recruit export receptors, yet with a lower affinity. In order to evaluate whether regulation of the interaction between RBPs and the export receptor is sufficient to retain aberrant mRNAs, we conducted a set of simulations on export of mRNAs with different affinities of RBPs to NXF1/NXT1. Two mRNA sequences of length 2.2 kb with different number of export receptor binding sites (9 and 12) are studied. Nuclear population of NXF1/NXT1 is varied across simulations, while the affinity of RBPs to export receptor is normal, reduced by 10-fold, or reduced by 100-fold. Based on experimental observations, mRNAs that are not fully processed (e.g. not spliced) are modelled as transcripts that have RBPs with a lower affinity to export receptor (Huang et al. 2004). Hereafter, these mRNAs are called aberrant mRNAs, while normally processed mRNAs that have RBPs with normal affinity to NXF1/NXT1 (Teplova et al. 2011) are termed normal mRNAs.

Considering that a typical mRNA of 2.2 kb length is expected to have an average of 9 binding sites for the export receptor (Sakharkar et al. 2004), our first set of simulations is performed on an mRNA sequence with 9 RBPs (as binding sites for the export receptor).

Percentage of successful mRNA export events for different expression levels of the export receptor within the nucleus is shown in Figure 3. Higher concentration of export receptor slightly increases the export of both the normal (Figure 3, gray plot) and the aberrant mRNA (Figure 3, pink plot). Export of aberrant mRNA reaches a plateau at approximately 7%, while export of normal mRNA slightly increases and reaches ~20%, which is in agreement with previously reported computational and *in vivo* export rates (Azimi et al. 2014; Siebrasse et al. 2012). On average, the percentage of successful export events of normal mRNA is three times that of aberrant mRNA (Figure 3-boxplot). Therefore, a lower affinity of RBPs (bound to aberrant mRNAs) to the export receptor leads to a considerable retention of aberrant mRNAs, while allowing normal mRNAs to be exported efficiently. However, this should be further evaluated for other configurations of mRNAs as well.

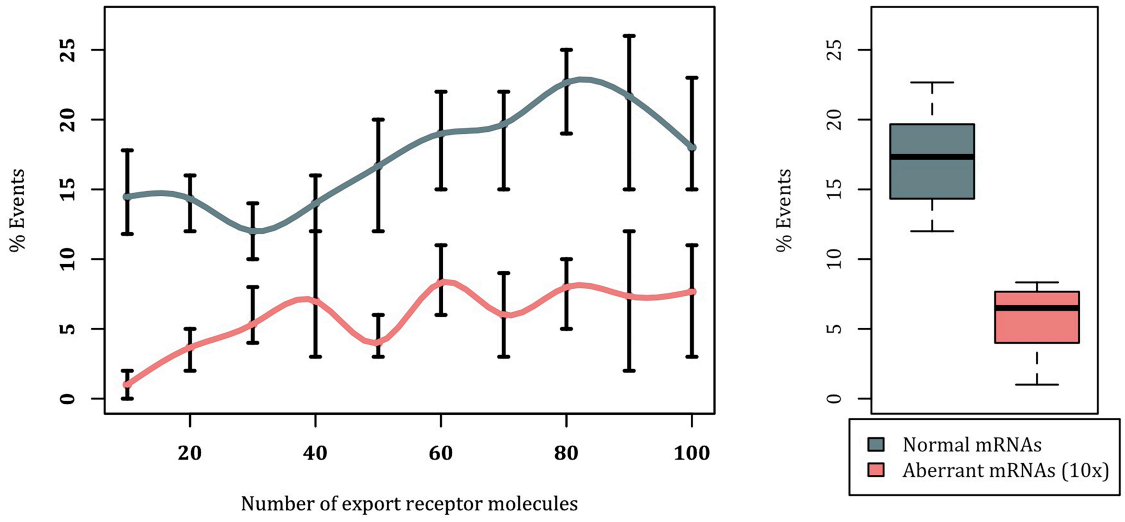

Figure 3: Export rate of an mRNA with 9 RBPs in the absence of the interaction between Tpr and RBPs. Left: Percentage export of mRNA versus export receptor copy number (varied from 10 to 100) for normal affinity between RBPs and NXF1/NXT1 (gray) and 10-fold decreased affinity (pink). For each data point, 100 replicates of the simulation are generated and analysed. The 100-replicate simulations are repeated 3 times. Error bars show the range of values obtained for each data point, while the data point itself is the averaged value among all the 3 repetitions of the 100-replicate simulations. It is clear that, regardless of the export receptor population, aberrant mRNAs cannot reach the normal mRNA export percentage. Right: Boxplot showing average successful export percentage across different NXF1/NXT1 copy numbers. In average, the successful export percentage of the normal mRNA is three times that of the aberrant one.

Second set of simulations was conducted on an mRNA sequence of the same length but higher number of export receptor binding sites (12 instead of 9). Percentage of successful export events with respect to the population of export receptor using different affinities between RBPs and NXF1/NXT1 is shown in Figure 4. As can be seen, the behaviour is significantly different from the previous experiment. The normal mRNA yields a successful export percentage of ~83%, in agreement with our previous mRNA export study (Figure 4, gray plot) (Azimi et al. 2014). Interestingly, regardless of NXF1/NXT1

copy number (except for the first data point), lowering the affinity of RBPs to NXF1/NXT1 by 10-fold for aberrant mRNAs (Figure 4, pink plot) would not inhibit efficient mRNA export, resulting in the same percentage of export events as the normal mRNA (Figure 4-boxplot). Nonetheless, further decrease of affinity, i.e. 100-fold decrease (see Figure 4, cyan), substantially decreases the export rate, regardless of export receptor copy number. Collectively, our results demonstrate that while regulation of the affinity between RBPs and export receptor (via phosphorylation of the RBPs) could retain aberrant mRNAs with average number of export receptor binding sites, it is not sufficient to retain all configurations of aberrant mRNAs.

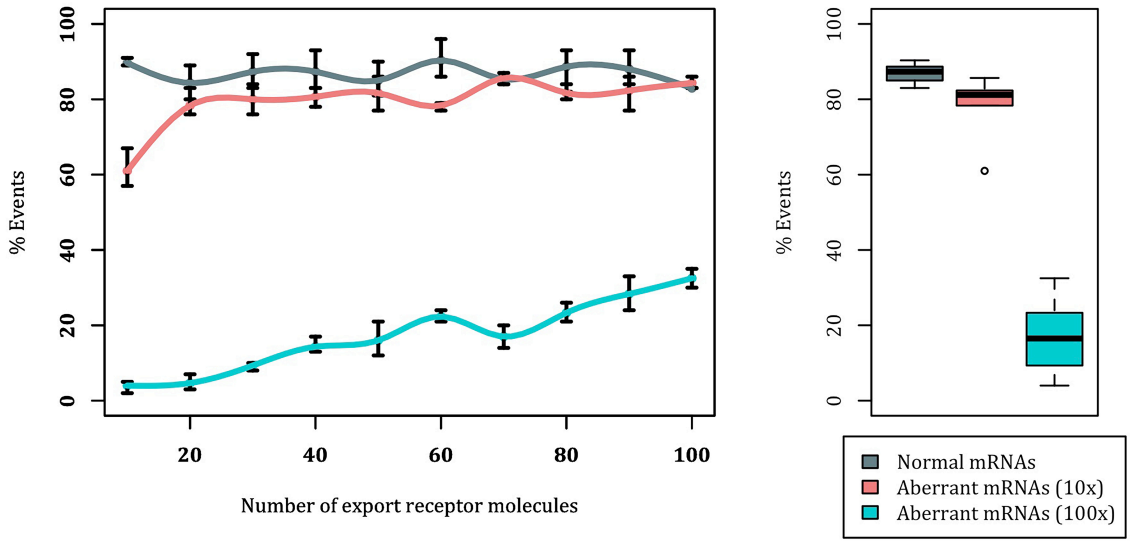

Figure 4: Export percentage of an mRNA with 12 RBPs in the absence of the interaction between Tpr and RBPs. Left: Successful export percentage of mRNA versus export receptor copy number (varied from 10 to 100) for normal affinity between RBPs and NXF1/NXT1 (gray), 10-fold decreased affinity (pink), and 100-fold decreased affinity (cyan). For each data point, 100 replicates of the simulation are generated and analyzed. The 100-replicate simulations are repeated 3 times. Error bars show the range of values obtained for each data point, while the data point itself is the averaged value among all the 3 repetitions of the 100-replicate simulations. Regardless of the copy number of NXF1/NXT1 molecules, except for the first data point, aberrant mRNAs that have 10-fold decreased affinity RBPs are transported as efficient as normal mRNAs. Right: Boxplot showing average successful export percentage across different export receptor copy numbers.

Lower affinity of RBPs to the export receptor enables Tpr to distinguish and retain aberrant mRNAs.

As the next step in developing our minimal model of the mRNA QC, the interaction between Tpr and RBPs is included in the model. An mRNA of length 2.2 kb with 12 RBPs (as the configuration that resulted in the largest number of aberrant mRNA export events in the absence of the interaction between Tpr and RBPs (Figure 4)) is simulated. The percentage of successful export events of the mRNA is shown in Figure 5. Interestingly, regardless of the population of NXF1/NXT1, the weak interaction between

Tpr and RBPs efficiently distinguishes aberrant mRNAs and prevents their export (Figure 5, pink bars), while allowing normal mRNAs to be exported properly (Figure 5, gray bars). This confirms our hypothesis that a lower affinity of RBPs to the export receptor, along with the interaction between Tpr and RBPs is the key to hinder the export of aberrant mRNAs.

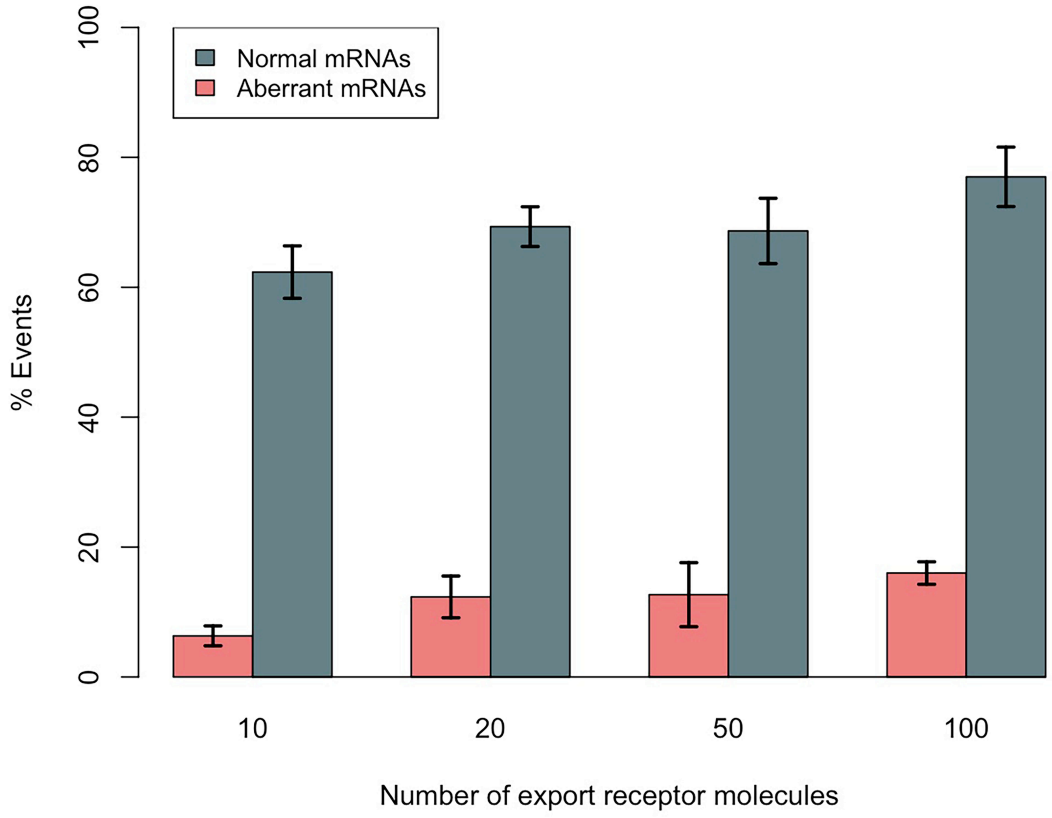

Figure 5: Effect of the interaction between Tpr and RBPs on the export of normal and aberrant mRNAs of length ~2.2 kb with 12 RBPs. Four different populations of export receptors are considered inside the nucleus, i.e. 10, 20, 50, and 100 molecules. The interaction between Tpr and RBPs efficiently hinders export of aberrant mRNAs (pink), while allowing proper transport of normal mRNAs (gray).

mRNA QC is a length-dependent mechanism

In order to understand whether mRNA length plays a role in the QC process, we repeated the previous study by simulating a shorter mRNA sequence of length ~500 b with the same density of RBPs (number per unit length) along its length. The percentage of export events of the mRNA with respect to NXF1/NXT1 copy number is presented in Figure 6. Although aberrant mRNAs are relatively retained, the QC mechanism is not as efficient as it was in the case of longer mRNAs. Therefore, length of the mRNA is a determining factor in this process. Of note, normal mRNAs are still being exported efficiently.

mRNA compaction at the nuclear basket is the reason for the length-dependency of the QC mechanism

Next, we sought to understand why mRNA length affects the QC process. The rate-limiting step in transport of mRNA molecules is suggested to be the at the nuclear basket, where the mRNA requires a considerable time to achieve an optimal configuration to initiate transport through the pore (Azimi et al. 2014; Siebrasse et al. 2012; Grünwald & Robert H Singer 2010). Therefore, since the quality control step is located at the nuclear basket, its length-dependent nature could be related to the difference in the optimal configuration of differently-sized mRNAs. Therefore, we conducted a set of simulations where two mRNAs of length 2.2 kb and 500 b (with the same density of RBPs) were simulated and their location and end-to-end distance were monitored throughout the simulation. The average end-to-end distance of both mRNAs is presented in Figure 7 with respect to their distance from the central channel of the NPC. As the longer mRNA approaches the nuclear basket, it requires to form a more compact conformation to be able to thread into the NPC (Azimi et al. 2014), which is a time-consuming process. In contrast, the short mRNA does not show any compaction in size as it reaches the NPC. Measuring the residence time of the long and short mRNAs inside the nuclear basket also shows that the short mRNA spends about 35% less time compared to the long one. Therefore, one could conclude that since longer mRNAs spend more time at the vicinity and inside the nuclear basket to form an optimal configuration for export, Tpr has more time to check their maturity and reject them in the case of identifying an aberrant mRNA.

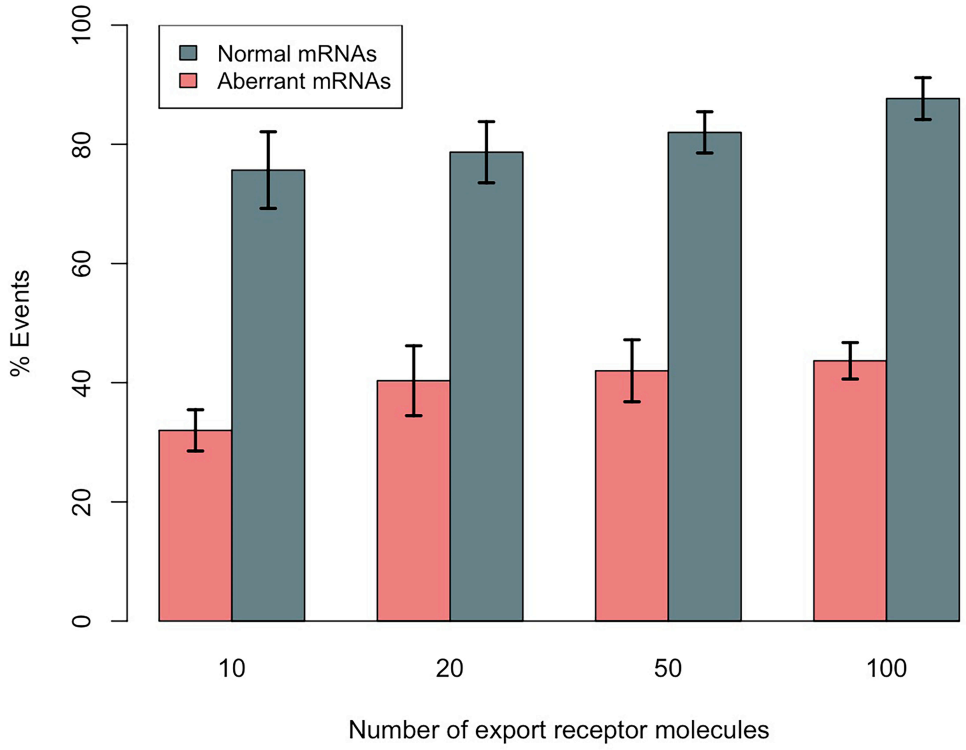

Figure 6: Effect of quality control protein Tpr on the export of normal and aberrant mRNAs of length 500 b with 3RBPs (similar number of export receptor binding sites per unit length as the mRNA simulated in the previous section). Four different populations of export receptors are considered inside the nucleus, i.e. 10, 20, 50, and 100 molecules. Aberrant mRNAs are relatively retained compared to normal mRNAs. However, it is clear that QC mechanism is not as efficient as it is in the case of longer mRNAs (Figure 5). Therefore, length of mRNA is a determining factor in mRNA QC.

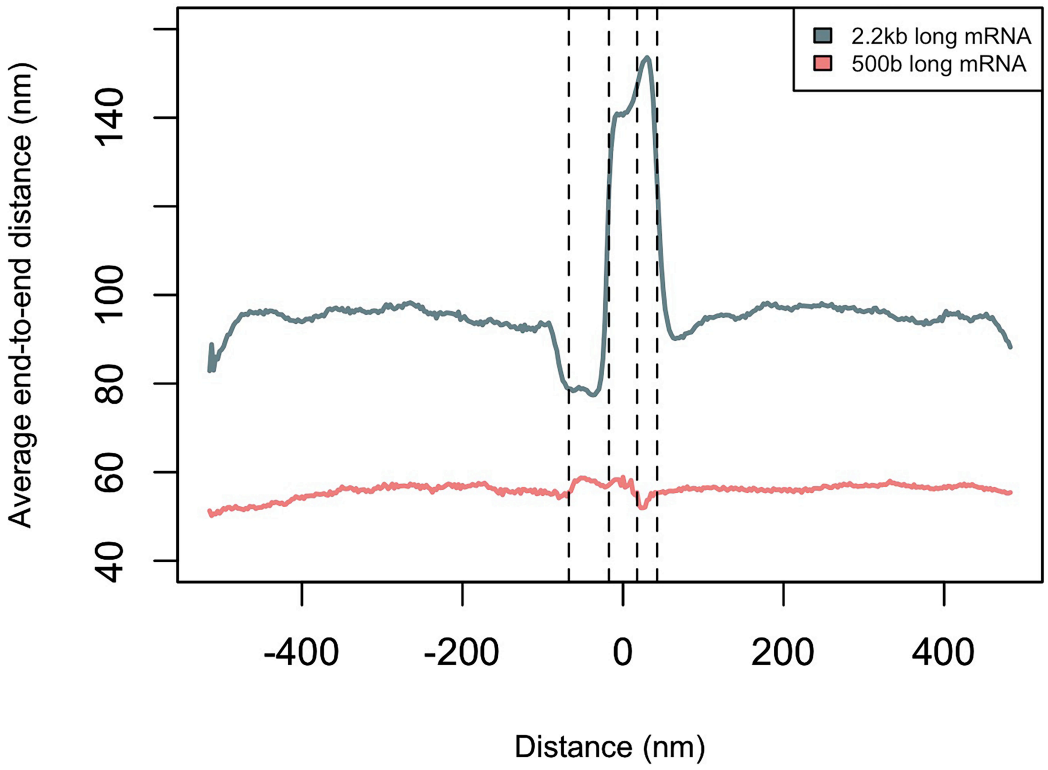

Figure 7: The average end-to-end distance of mRNAs averaged over 100 simulations with respect to their distance from the central channel of the NPC. Two mRNAs are simulated with lengths 500 b and 2.2 kb. The x-axis represents the position along the axis perpendicular to the nuclear envelope, with x = 0 set at the center of the central channel of the NPC. From left to right, the dashed lines represent the distal edge of the nuclear basket, the nuclear edge of the central channel, the cytoplasmic edge of the central channel, and the distal edge of the cytoplasmic filaments, respectively. The end-to-end distance of the longer mRNA is significantly dropped as it approaches the nuclear basket, to form a more compact conformation to be able to pass through the NPC (Azimi et al. 2014). The short mRNA, on the other hand, does not show any compaction in size as it reaches the NPC. This difference explains the length-dependent nature of mRNA QC process.

The interaction between Tpr and RBPs is optimized for an efficient retention of aberrant mRNAs

In order to demonstrate the role of the interaction between Tpr and RBPs, we compared the export rate of aberrant mRNAs with respect to the change in the affinity of this interaction. Two different populations of export receptor (20 and 100 molecules) and two different lengths of mRNA (500 b and 2.2 kb) were simulated. The experimentally

measured affinity of ~1μM is optimized, as it is the weakest interaction that leads to the minimum export rate of aberrant mRNAs (in other words the highest retention of aberrant mRNAs). Higher affinities lead to the same rate and lower affinities result in considerably higher export rates of aberrant mRNAs (Figure 8). Therefore, our results demonstrate that the interaction between Tpr and RBPs is optimized for the most efficient retention of aberrant mRNAs.

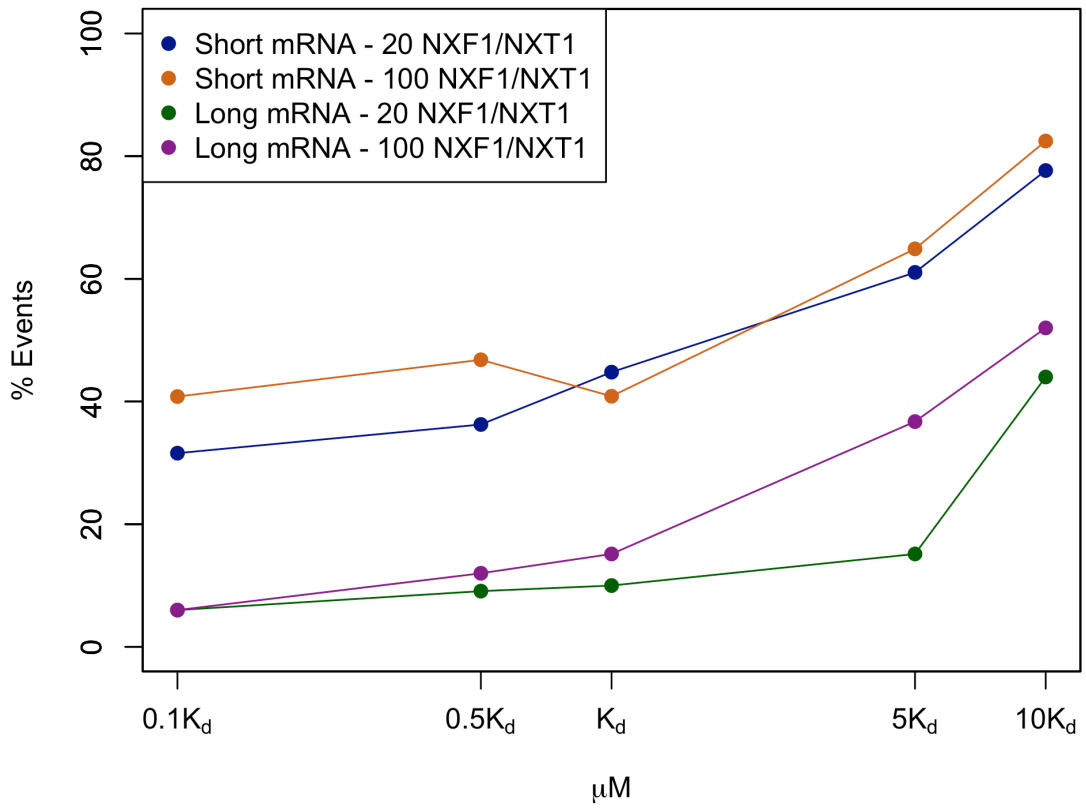

Figure 8: Successful export percentage of aberrant mRNAs with respect to the affinity between Tpr and RBPs. Dissociation constant of Tpr to RBPs is changed from 0.1Kd to 10Kd , where Kd is the experimentally measured value of the dissociation constant, to demonstrate how efficient this interaction is in retaining aberrant mRNAs. Two different populations of NXF1/NXT1 dimers (20 and 100) and two different mRNA lengths (500 b and 2.2 kb) are simulated. Dissociation constants smaller than the reported value (~1μM) almost yield the same export percentage, while larger dissociation constants result in significantly higher export percentage of aberrant mRNAs. Therefore, the ~1μM dissociation constant is optimized such that, with the weakest interaction possible, it efficiently retains aberrant mRNAs.

Discussion

Despite numerous studies conducted to understand the mRNA QC procedure, the underlying mechanism is still unclear (Hackmann et al. 2014). While several different proteins are identified to be involved in retention of aberrant mRNAs, no detailed explanation for how aberrant mRNAs are distinguished is presented so far. It is not even clear whether normal mRNAs are selected to be exported (selection model), aberrant

mRNAs are retained inside the nucleus (retention model), or a combination of both strategies is employed in eukaryotic cells (Bonnet & Palancade 2015).

In this work, we developed a computational model to identify the minimal system required to distinguish and retain aberrant mRNAs. Our results demonstrate that mRNA QC is primarily achieved by a cooperation between two key interactions: 1) the interaction between RBPs and the export receptor and 2) the interaction between the nuclear basket associated Tpr and RBPs. We showed that regulation of the interaction between RBPs and the export receptor enables Tpr to distinguish and retain aberrant mRNAs. The interaction between RBPs and the export receptor is regulated by their phosphorylation state. Npl3, one of the yeast RBPs, is phosphorylated when recruited to pre-mRNAs; and upon successful processing of mRNAs becomes dephosphorylated (Huang & Steitz 2005). Hypophosphorylated form of 9G8 and ASF/SF2 (two metazoans RBPs) that is bound to normal mRNAs also have a higher affinity to the export receptor (TAP) compared to their hyperphosphorylated form that is bound to aberrant mRNAs (Huang et al. 2004). Therefore, RBPs bound to aberrant mRNAs have lower affinity to the export receptor, compared to the RBPs bound to normal mRNAs. This slight difference, as demonstrated in this work, is sufficient for Tpr to distinguish and retain aberrant mRNAs. It was previously suggested that formation of aberrant mRNAs might be signalled via a yet-to-be-identified intron-bound protein to retain the mRNA (Bonnet & Palancade 2015). However, our results suggest that mRNA QC is achieved by cooperation of regulated stochastic interactions between the involved proteins rather than deterministic switch-like properties. Nevertheless, the system we identified as the minimal requirement for mRNA QC is not perfect in retaining aberrant mRNAs, specifically for short mRNAs (Figure 6). Therefore, the hypothesis that some yet-unknown proteins might signal the formation of aberrant mRNAs is still valid, which could further improve mRNA QC efficiency.

Our results highlight the effect of the density (number per unit length) of export receptor binding sites on mRNA sequence, and the length of the mRNA on mRNA export and QC. Regarding the length-dependent behavior of mRNA QC, we demonstrated that, it is more challenging for Tpr to retain short aberrant mRNAs compared to longer ones. The reason for this length-dependency is the more time that longer mRNAs spend in the vicinity and inside the nuclear basket to form their optimal configuration for export. As a result, Tpr is able to capture long aberrant mRNAs, while short aberrant mRNAs can quickly escape this step as they do not require a compaction in their size. Interestingly, it has been recently shown that flow-driven translocation of polymers through nanochannels is also length-dependent (Ledesma-Aguilar et al. 2012). Moreover, we demonstrated that the affinity between Tpr and RBPs is optimized to efficiently retain aberrant mRNAs. This interesting finding stresses on the significant role of binding affinities in such a mechanism that is primarily driven by stochastic interactions.

As mentioned before, our model is a minimal system for the QC of mRNAs and we have primarily explored the retention model. There are several other aspects to this process as well. However, we argue that inclusion of other factors will further refine the performance of mRNA QC. In fact, given the imperfect retention of aberrant mRNAs in some cases in our simulations, we expect other factors to be involved to improve the

performance of the system. In our model, Tpr has the same affinity to RBPs bound to either normal or aberrant mRNAs, because it is not known whether phosphorylation state of RBPs affect their interaction with the Tpr. However, in the case that Tpr has different affinities to RBPs bound to normal and aberrant mRNAs, it is expected to interact more strongly with RBPs bound to aberrant mRNAs, which will further improve the performance of our model to retain aberrant mRNAs. As another example, whether Tpr interacts with the complex of RBPs and the export receptor, which is required to evaluate the selection model, has yet to be investigated. Mlp1 (yeast homologue of Tpr) is found in complex with various mRNA export machinery proteins including Mex67 (yeast homologue of NXF1) (Niepel et al. 2013). Moreover, Mex67 has been reported to indirectly interact with Mlp1 (Vinciguerra et al. 2005). However, whether the indirect interaction between the export receptor and Mlp1 is achieved through RBPs is not known. Nevertheless, in the case this interaction (Tpr with the complex of RBP-export receptor) exists, it is expected that the formation of RBP-export receptor complex modulates the interaction between Tpr with RBPs in order not to disrupt the export of normal mRNAs, which, again, improves the retention performance of the system. Therefore, it is conceivable to suggest that the retention and selection models are both in place to maximize the performance of mRNA QC.

Collectively, we demonstrated that regulation of the affinity of RBPs to the export receptor enables Tpr (Mlp1) to recognize and retain aberrant mRNAs.. However, this minimal system was not successful to perfectly retain aberrant mRNAs in some situations, justifying the involvement of several other factors and co-factors in mRNA QC to improve its performance in recognizing aberrant mRNAs.

Chapter 4:

Dissecting molecular interactions involved in mRNA Quality Control using all-atom Molecular Dynamics

Molecular mechanisms of the interaction between the RNA-binding protein Nab2 and the nuclear basket protein Mlp1

Introduction

Nuclear export of messenger ribonucleic acid (mRNA) transcripts is an essential step in regulation of gene expression in eukaryotic cells (Carmody & Wente 2009). Many RNA molecules including tRNA, microRNA (miRNA), small nuclear RNA (snRNA), and rRNA are transported via a karyopherin (Kap)-mediated pathway, whereas bulk of mRNA export is mediated by heterodimeric export receptors, namely Mex67-Mtr2 in yeast, TAP-p15 or NXF1-NXT1 in metazoans. While Kap-mediated export requires RanGTPase cycle for directionality, bulk mRNA export does not directly depend on the RanGTPase system (Natalizio & Wente 2013). Transcription of mRNA from deoxyribonucleic acid (DNA) is followed by several processing and packaging steps, eventually forming an export-competent messenger ribonucleoprotein (mRNP) to be exported to the cytoplasm through the nuclear pore complexes (NPCs) (Köhler & Hurt 2007; Luna et al. 2008). mRNAs are spliced and undergo 5'-end capping, 3'-end cleavage and polyadenylation processes inside the nucleus (Tutucci & Stutz 2011). In addition, they are co-transcriptionally loaded with several RNA-binding proteins (RBPs), such as Npl3 by RNA polymerase II and Gbp2 and Hrb1 via the THO complex, which play a key role in transport of mRNAs. (Dreyfuss, V. Narry Kim, et al. 2002; Hurt et al. 2004; Tutucci & Stutz 2011).

Packaging and processing of mRNAs are quality controlled at different stages of RNA biogenesis via various sophisticated, yet efficient, mechanisms in eukaryotic cells (Eberle & Visa 2014; Tutucci & Stutz 2011; Porrua & Libri 2013). One of the specific steps of mRNA surveillance pathway occurs at the entry of the NPC, which involves various types of proteins ranging from RBPs to nuclear pore associated proteins including yeast proteins Nab2, Gbp2, Hrb1, Npl3, Mlp1, Mlp2, Nup60, Pml1, Pml39, and Esc1 (Galy et al. 2004; Palancade et al. 2005; Hackmann et al. 2014; Lewis et al. 2007; Green et al. 2003). While suitably processed mRNAs, decorated by RBPs, will be primed for export through the NPC by recruiting heterodimeric export receptors, the faulty mRNAs would be retained inside the nucleus and will be marked by the TRAMP (Trf4/Air2/Mtr4 Polyadenylation) complex for degradation at the nuclear exosome (Hackmann et al. 2014; Tutucci & Stutz 2011). The intricate nature of this pathway requires several cross-interactions between the involved factors, leading to the recognition of aberrant mRNAs and degradation at the exosome to prevent them from engaging in translation (Chlebowski et al. 2013; Niño et al. 2013).

Of all proteins involved in this exquisite process, the nuclear basket-associated protein Myosin-like protein-1 (Mlp1), or Tpr in humans, (Frosst et al. 2002; Krull et al. 2004) has been suggested to play a key role (Fasken et al. 2008; Green et al. 2003; Hackmann et al. 2014). Although Mlp1 is not required for cell viability (Strambio-de-Castillia et al. 1999; Kölling et al. n.d.), deletion of MLP1 leads to impaired retention of intron-containing mRNA transcripts (Galy et al. 2004). The role of Mlp1 in mRNA quality control is further supported by studies that demonstrate the interaction between Mlp1 with various yeast RBPs, including Nab2 (Green et al. 2003; Fasken et al. 2008; Grant et al. 2008), Gbp2 and Hrb1 (Hackmann et al. 2014), and Npl3 (Green et al. 2003). Moreover, our recent study on the overall dynamics of mRNA quality control highlighted the significance of the interaction between RBPs and Mlp1 in the process of mRNA

quality control (Soheilypour & Mofrad 2016). Mlp1 is suggested to act both as a quality control checkpoint and a docking site for passing mRNPs, enabling the complex to undergo the necessary remodeling steps for transport (Tutucci & Stutz 2011).

The interaction between Nab2, one of the Saccharomyces cerevisiae RBPs, and Mlp1 has previously been explored through both in vitro and in vivo experiments (Fasken et al. 2008; Green et al. 2003). Nab2 is a zinc finger protein, essential for cell viability, and required for the export of poly(A) RNA transcripts (Anderson et al. 1993; Green et al. 2002; Kelly et al. 2007; Wilson et al. 1994). Nab2 shuttles between the nucleus and the cytoplasm and its lifecycle is regulated via three functional domains (Duncan et al. 2000), namely: the N-terminal domain (residues 1-97) that is essential for the export of mRNA and Nab2 itself into the cytoplasm (Marfatia et al. 2003); an RGG domain (residues 201-265) that serves as a nuclear localization signal (NLS) for the import of Nab2 into the nucleus via Kap104 (Lee & Aitchison 1999); and a zinc finger domain (residues 262-477) specific binding to polyadenosine RNAs (Marfatia et al. 2003; Kelly et al. 2007; Fasken et al. 2008). The N-terminal domain of Nab2 (Nab2-N) is initially found to interact with a 385-residue region of Mlp1 (residues 1490-1875) (Green et al. 2003). Nab2-N is both necessary and sufficient for this interaction (Grant et al. 2008). However, a more recent study has refined the Nab2-binding domain of Mlp1 to a 183-residue region (Mlp1-C; residues 1586-1768) (Fasken et al. 2008). These studies identified a hydrophobic patch within the Nab2 N-terminal domain, centered on F73, which was suggested as being critical for the interaction between the two proteins. However, the underlying molecular mechanism of Mlp1-Nab2 complex formation and the role of F73 in this process are still elusive.

In our recent publications on nucleocytoplasmic transport, we utilized various computational techniques across different scales to study key atomic-level interactions to larger scale dynamics of the system (M Peyro et al. 2015; Mohaddeseh Peyro, Soheilypour, Ghavami & Mohammad R.K. Mofrad 2015; Azimi & Mofrad 2013; Jamali, Jamali, Mehrbod & M. R. K. Mofrad 2011; Wolf & Mofrad 2008; Jahed et al. 2016; Soheilypour et al. 2016; Zhao et al. 2014; Moussavi-Baygi & Mofrad 2016). In particular, our recent studies on mRNA export and quality control mechanisms (Azimi et al. 2014; Soheilypour & Mofrad 2016) motivated us to explore Mlp1-Nab2 complex formation in greater detail as a critical step in the quality control process. Here, we employed an integrated computational approach to study the pivotal interaction between Mlp1 and Nab2 in mRNA export and quality control processes. First, we conducted a sequence homology modeling to predict the structure of Mlp1-C and then used it for a docking analysis on Nab2-N and Mlp1-C to identify most promising orientations of the molecules for complex formation. Following structural refinements, we carried out molecular dynamics (MD) simulations to explore the detailed binding mechanism of Mlp1 and Nab2. We also investigated the effect of experimentally tested mutations on the F73 residue (Green et al. 2003; Fasken et al. 2008) in order to both validate the model and further elucidate the role of F73 in Mlp1-Nab2 binding.

Materials and Methods:

Molecular dynamics simulations were conducted using Gromacs (Pronk et al. 2013), and CHARMM27 force field (Klauda et al. 2005; Feller & MacKerell 2000). Mutations were modeled using VMD 1.9.1 (Humphrey et al. 1996). Crystal structure of N-terminal domain of Nab2 (PDB ID: 2V75) was used in the simulations (Grant et al. 2008). Crystal structure of the C-terminal domain of Mlp1 is not available, presumably because it is partly unstructured, similar to its human homologue Tpr (Byrd et al. 1994). Therefore, we used various secondary and 3D structure prediction servers including Phyre2, I-Tasser, RaptorX, Psi-Pred, and HHPred (Kelley & Sternberg 2009; McGuffin et al. 2000; Roy et al. 2010; Söding 2005; Wang et al. 2011) to build the protein structure of the C-terminal domain of Mlp1 by homology modeling. ZDock 3.0.2 server was used for docking simulations (Pierce et al. 2014). It should be noted that, prior to performing docking analyses, Nab2-N and Mlp1-C molecules were equilibrated for 20 ns in a water box using MD simulations. Top 10 solutions of the docking analysis were considered as potential orientations for the interaction between the two molecules. As mentioned earlier, it was experimentally observed that F73 is essential for this interaction. Therefore, the solutions in which the hydrophobic helix of Nab2 (where F73 is located) was not facing Mlp1-C (solutions 2, 4, 5, 9, and 10) were excluded. Molecular dynamics simulations were conducted to identify the most favorable orientation for binding.

Each system was placed in a water box with the TIP3P water molecules, a 22A margin, and counter-ions were added to neutralize the extra charge of the system and the ion concentration was set to 150mM NaCl. Periodic boundary conditions were used in simulations. Each system was minimized for 5000 steps with a 2 fs timestep. Subsequently, each system was simulated for 10ns, 1 ns for the equilibration and 9 ns for the production runs. Energy profiles of the 10 ns simulations were compared across different orientations of interactions. The best orientation was chosen as the one with the highest absolute energy during the 9 ns run.

For the main run, since the predicted structure for Mlp1-C is partly unordered, we ran long 100 ns equilibration simulations, to ensure that the complex has reached its minimum energy. After the equilibration, each complex (wild-type/mutant) was simulated for an extra 20 ns, and each simulation was repeated three times to account for the stochasticity in the system. The Bio3D R package was used for data visualization and dynamic cross correlation analysis (Skjærven et al. 2014), while molecular visualization were performed via Visual Molecular Dynamics (VMD) 1.9.1. The distance between the two helices of Nab2 was calculated by measuring the distance between the center of mass of the two helices.

Results

Homology modeling suggests a relatively large unordered region at the C-terminus of Mlp1

The structure of Mlp1-C was constructed via homology modeling. The Nab2-binding domain of Mlp1 was mapped to a 183-residue region within its C-terminal domain

(Mlp1-C; residues 1586-1768). Since the crystal structure of this region is not available, we used various secondary and 3D structure prediction tools, including Phyre2, I-Tasser, RaptorX, Psi-Pred, and HHPred (Kelley & Sternberg 2009; Wang et al. 2011; McGuffin et al. 2000; Roy et al. 2010; Söding 2005), to predict the 3D structure of Mlp1-C. I-Tasser predicted that the first 84 residues form helices, while the rest of the structure is unordered (the unordered region includes two very small helices). The confidence score is calculated to be -2.06 in a [-5, 2] range, and the TM-score is 0.47±0.15, with TM>0.5 predicting the correct topology (Zhang & Skolnick 2004). Therefore, the predicted model is a relatively reliable model. Phyre2 also predicted the first 86 residues form helices as well as some small helices in the rest of the sequence. The rest of the sequence was predicted to be unordered. The confidence measured for the first 86 residues was about 70% (Kelley & Sternberg 2009). Similarly, Psipred (McGuffin et al. 2000) predicted the first 86 residues as helices and the rest of the sequence as unordered, mostly with a high confidence level. RaptorX (Wang et al. 2011) predicted the first 89 residues form two long helices with a p-value of 2.06e-2, and the rest of the sequence as being unordered. Finally, HHpred also predicted the first 86 residues form helices and the rest to be unordered. The predicted secondary structure of the sequence (helices versus coils) is consistent among all servers, i.e. the first half formed two long helices, while the second half conformed an unordered structure. The predicted structure is also in agreement with previous reports on Tpr (human homologue of Mlp1) structure, which has a long coiled coil region followed by an unordered domain at the C-terminus (Byrd et al. 1994). In this study, the 3D structure predicted by Phyre2 server is used (Figure 1).

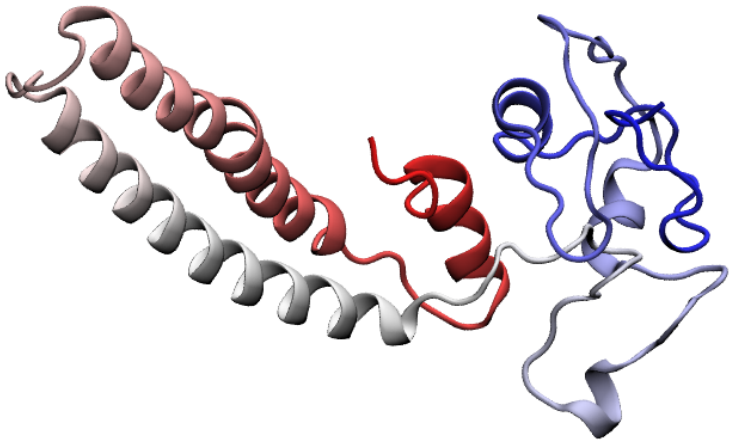

Figure 1: Predicted structure of the C-terminal domain of Mlp1 (residues 1586-1768) using the Phyre2 Web server (Kelley & Sternberg 2009). The structure is colored from the N-terminus (red) to the C-terminus (blue). The N-terminal domain is composed of two helices, while the C-terminus is mostly unordered.

Constructing the Mlp1-Nab2 complex

There is no structural information available on the Mlp1-Nab2 complex. Therefore we utilized molecular docking (ZDOCK server (Pierce et al. 2014)) to obtain a sample of potential binding modes. Both Mlp1-C and Nab2-N were equilibrated for 20 ns before docking studies. Docking results indicated that the unordered domain of Mlp1-C is likely

the binding site for Nab2-N (Figure 2). Out of the top 500 solutions, only a few suggested that Nab2-N interacts with structured regions of Mlp1-C.

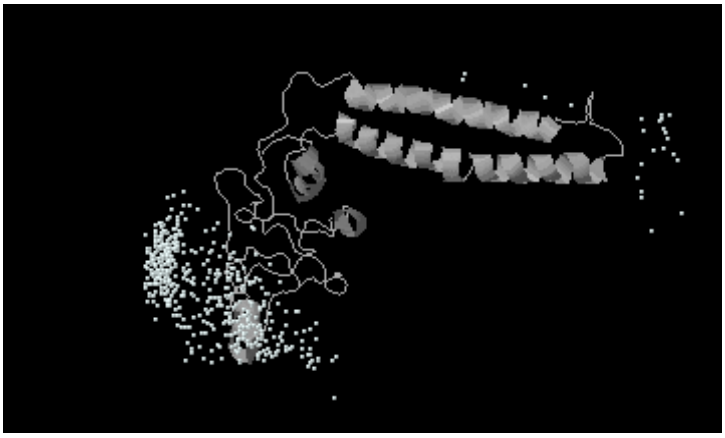

Figure 2: Top 500 solutions of docking analysis on Mlp1-C and Nab2-N. The whole structure of Mlp1-C is shown, while only center of masses of Nab2 in the docking solutions are presented by dots. The concentration of dots around the unordered region of mlp1 indicates that it is significantly more favorable for Nab2-N to interact with this region.

Are the predicted docking solutions reliable?

It is generally suggested that docking solutions are less reliable for unordered regions, as the structure is less stable and might experience significant conformational changes that would affect the docking results. We acknowledge this fact and present the following reasoning to suggest that our docking results are sufficiently reliable: Mlp1-C structure was equilibrated for 20 ns before docking simulations. During the equilibration process, no significant conformational changes were observed in the unordered domain of Mlp1-C as indicated by the radius of gyration (Figure 3) and root mean square deviation (RMSD) of the unordered region compared with the ordered region of Mlp1-C during the simulation (Figure 4).

Identifying the most preferred orientation for interaction

The top ten docking solutions were considered for further refinements. As shown by previous *in vitro* and *in vivo* studies, a hydrophobic patch on Nab2-N centered on F73 is essential for Mlp1-C binding (Green et al. 2003; Fasken et al. 2008). Based on this observation, five out of ten docking solutions were filtered out, as Nab2-F73 was not correctly facing Mlp1. The remaining five docking solutions are shown in Figure 5.

Although the molecular docking method provides geometrical insights into favorable orientation of the binding partners, it lacks the information regarding the dynamics and stability of the complex over time. Thereby, we equilibrated all five promising docking solutions (Figure 6) using MD simulations in order to examine the stability of each complex. The binding energies between Mlp1-C and Nab2-N were monitored throughout the last 9ns of simulations as shown in Figure . The interaction energy of solution 1 was clearly much stronger than the rest of the solutions (Figure 2), hence the solution 1 was considered as the most favorable complex and further studied through MD simulations.

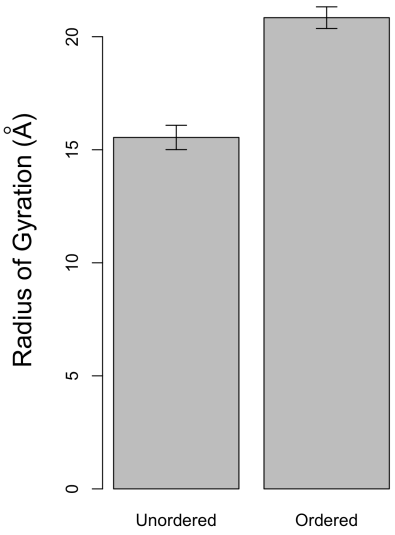

Figure 3: Radius of gyration of the unordered and ordered regions of Mlp1-C during equilibration. The standard deviation (shown by error bars) of the unordered region is similar to that of the ordered region, suggesting a relatively stable unordered conformation without considerable conformational changes.

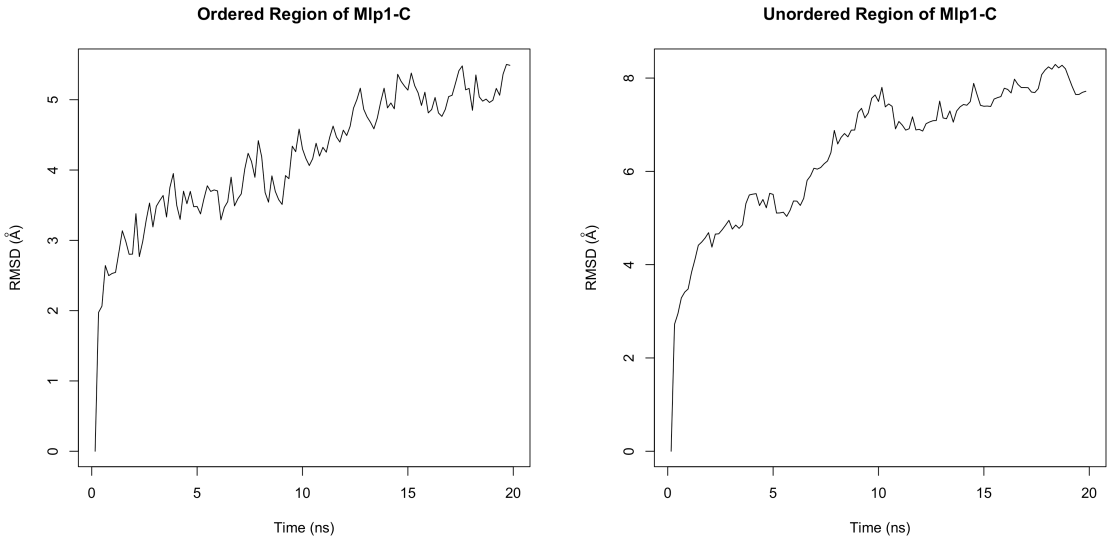

Figure 4: Root mean square deviation (RMSD) of the ordered and unordered region of Mlp1-C during equilibration. RMSD reaches a plateau for both domains indicating that the structure has reached equilibrium. Also, comparing RMSD of the ordered and unordered domains, it could be concluded that the unordered region does not significantly change conformation.

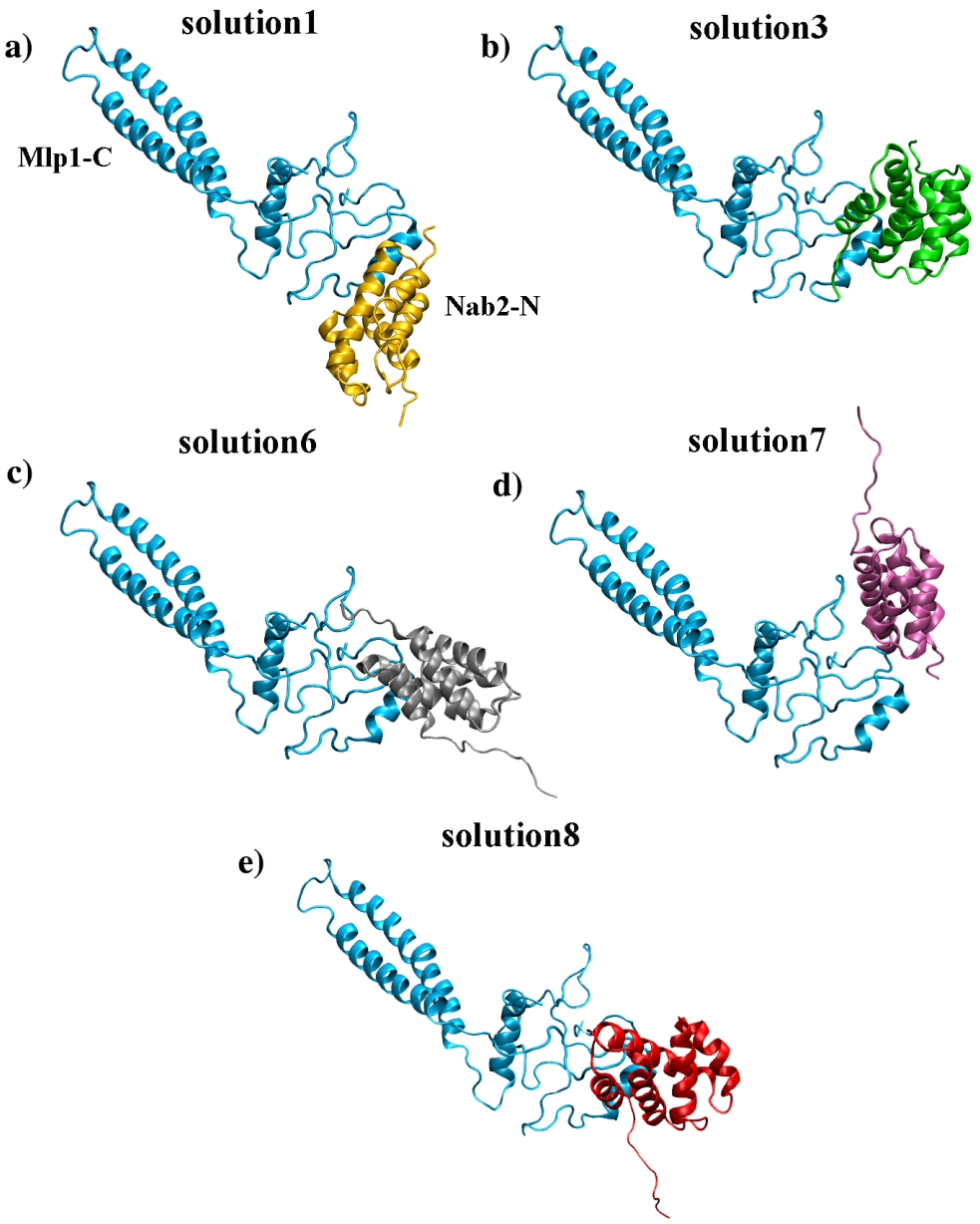

Figure 5: Potential binding orientations between Mlp1-C and Nab2-N. The top 10 solutions from docking of Mlp1-C and Nab2-N were considered out of which five solutions were excluded, as they did not satisfy experimental observation of Nab2-F73 being crucial for this interaction. In all solutions, Mlp1-C is shown in blue, while Nab2-N is shown in yellow (solution1), green (solution3), grey (solution6), magenta (solution7), and red (solution8).

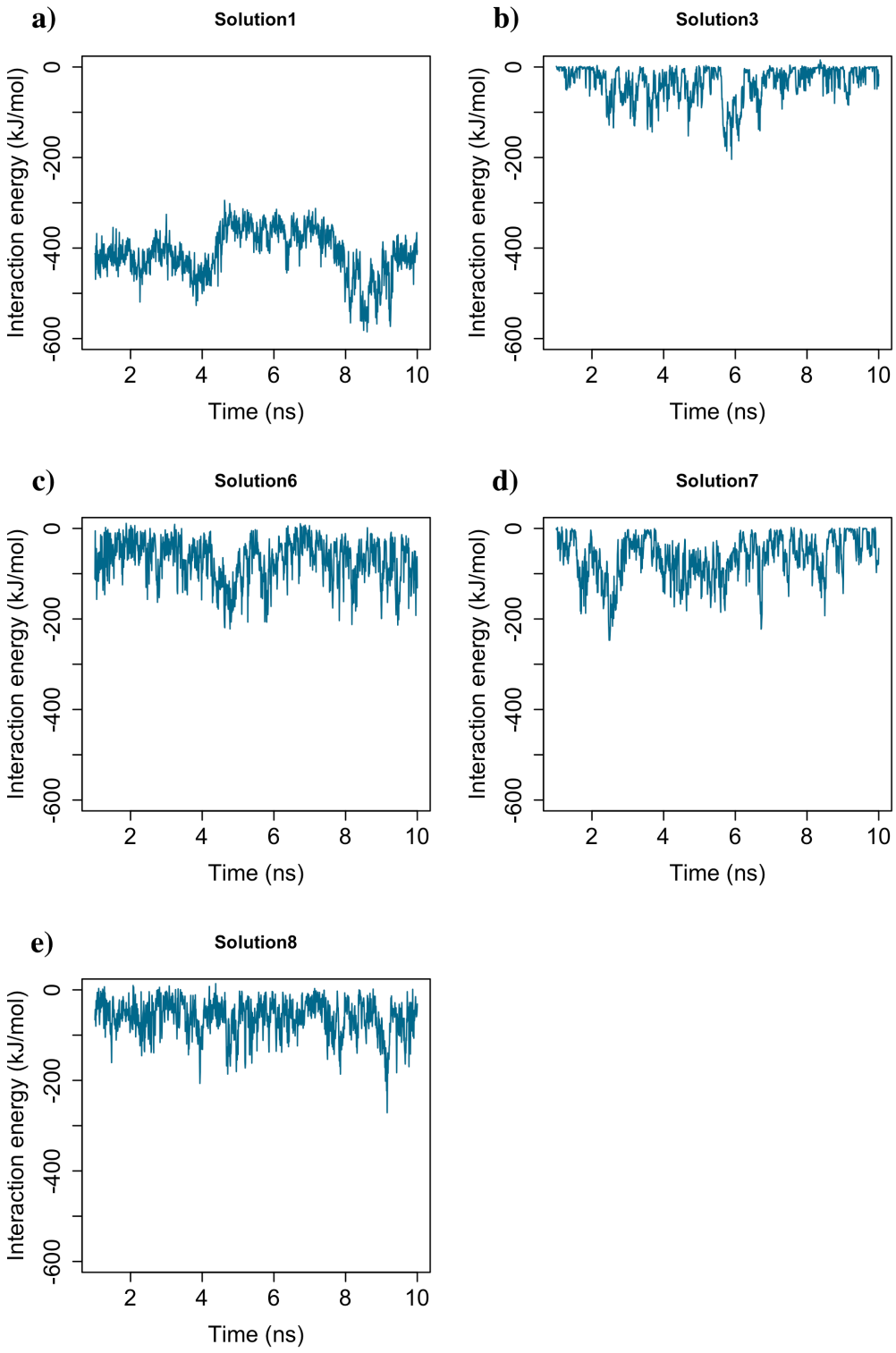

Figure 6: The interaction energies between Mlp1-C and Nab2-N in selected docking solutions (a-e) after 10 ns of simulation following equilibration. Solution1 showed the strongest binding energy .

Nab2-F73 is essential for the interaction of Nab2-N with Mlp1-C

In order to validate our computational protocol, i.e. structure prediction followed by docking simulations and all-atom MD simulations, and to further explore the significance of F73 in Mlp1-Nab2 complex formation, solution 1 was equilibrated both in the wild-type and Nab2-F73A mutant. Since the predicted structure for Mlp1-C was partially unordered, we first ran a single relatively long simulation (100 ns) to ensure that the complex has reached its equilibrated state. Subsequently, each complex (wild-type/mutant) was simulated for additional 50 ns, and repeated three times to account for the stochasticity in the system. The interaction energies between Mlp1-C and Nab2-N in the wild-type and mutant configurations are presented in Figure 7. The interaction energy in the wild-type complex is about three times stronger than that of the mutant, which verifies the substantial role of Nab2-F73 in this interaction as indicated by previous studies (Fasken et al. 2008) and validated our computational protocol for constructing the Mlp1-Nab2 complex. We further investigated the mechanisms by which F73 enhanced the strength of Mlp1-Nab2 complex as presented in the following section.

Nab2-F73 plays an indirect role in the interaction between Nab2-N and Mlp1-C

The interaction energy between Mlp1-C and Nab2-N during the 100 ns equilibration simulation was analyzed (Figure 8). Interestingly, only in the wild-type simulation, a dramatic increase in the binding energy occurred after 60 ns, from ~ -400 kJ/mol to ~ -1200 kJ/mol, which was associated with a major change in the electrostatic interactions between Mlp1-C and Nab2-N as well as a minor increase in hydrophobic interactions. The binding simulations revealed a conformational change in the wild-type complex that resulted in the substantial change of energy as discussed in the following section.

Interestingly, the energy between Nab2-F73 and Mlp1-C did not exhibit any significant change with a relatively constant interaction energy of ~ -40 kJ/mol (Figure 9), which suggests an indirect role for F73 in the binding. Simulations showed that Nab2-N consists of five helices out of which only two (helix4 containing F73 and helix5) were in direct contact with Mlp1-C as shown in Figure 10. The interaction energy between F73 and helix5 increases around 60 ns (Figure 11), implying that this interaction might be the starting point for downstream effects that lead to the binding between Mlp1-C and Nab2-N. Moreover, comparison of the interaction energies between helix4 and helix5 in the wild-type and mutant indicated that F73 had a considerable effect on the interaction between the two helices and most likely facilitated the binding between Mlp1-C and Nab2-N through interacting with helix5. The distance between helix4 and helix5 during the production run was maintained at a smaller value in the wild-type (12.61 ± 0.32 Å), compared to that of the mutant (13.51 ± 0.58 Å) (Figure 12).

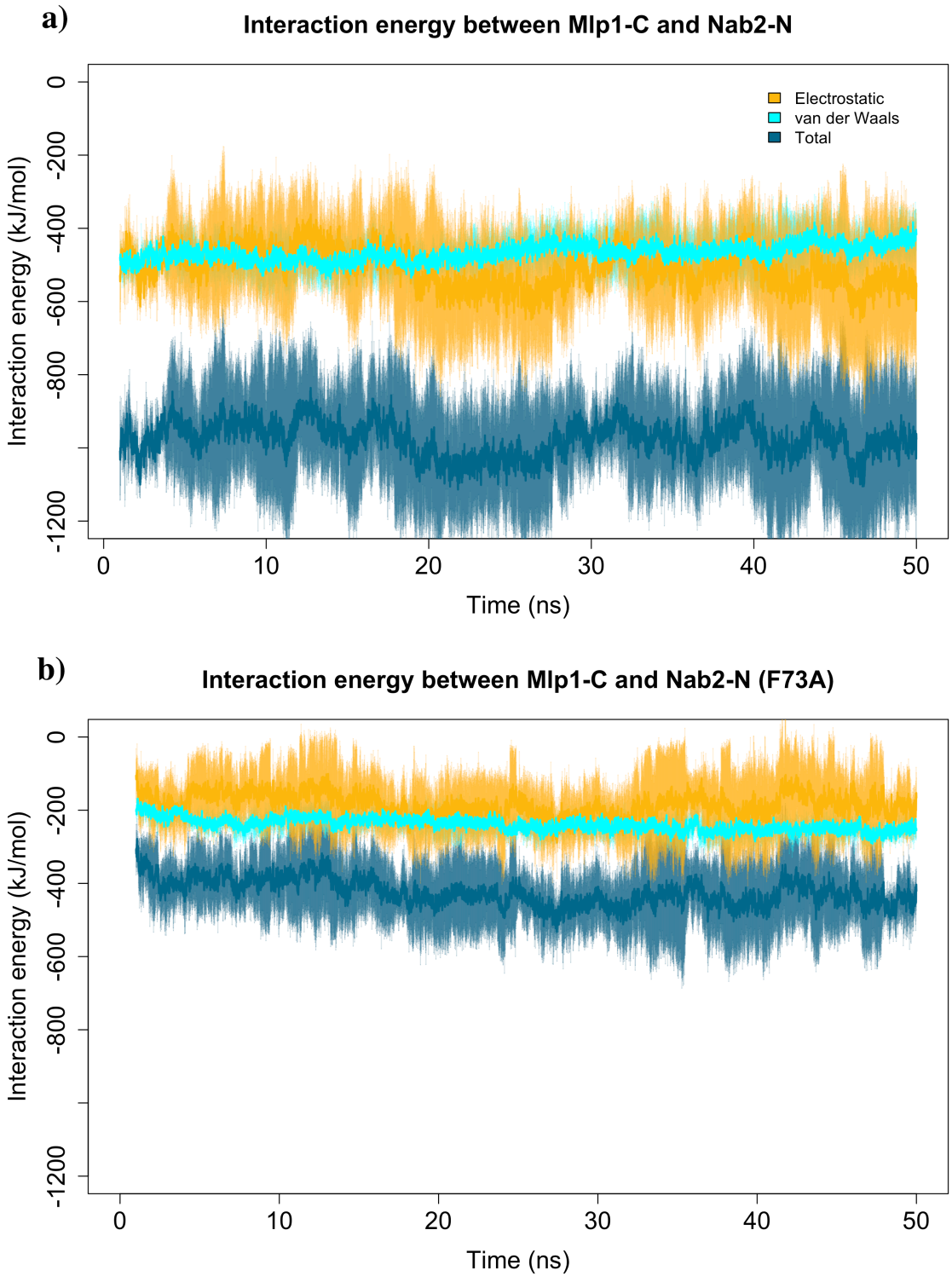

Figure 7: The interaction energies between Mlp1-C and Nab2-N for both wild-type and mutant. The solid lines show the average of the data points from the three simulations carried out for a) wild-type and b) mutant and the shaded area shows the standard deviation. The wild-type complex has interaction energy of

about three times that of the mutant complex, demonstrating the substantial role of Nab2-F73 in this interaction.

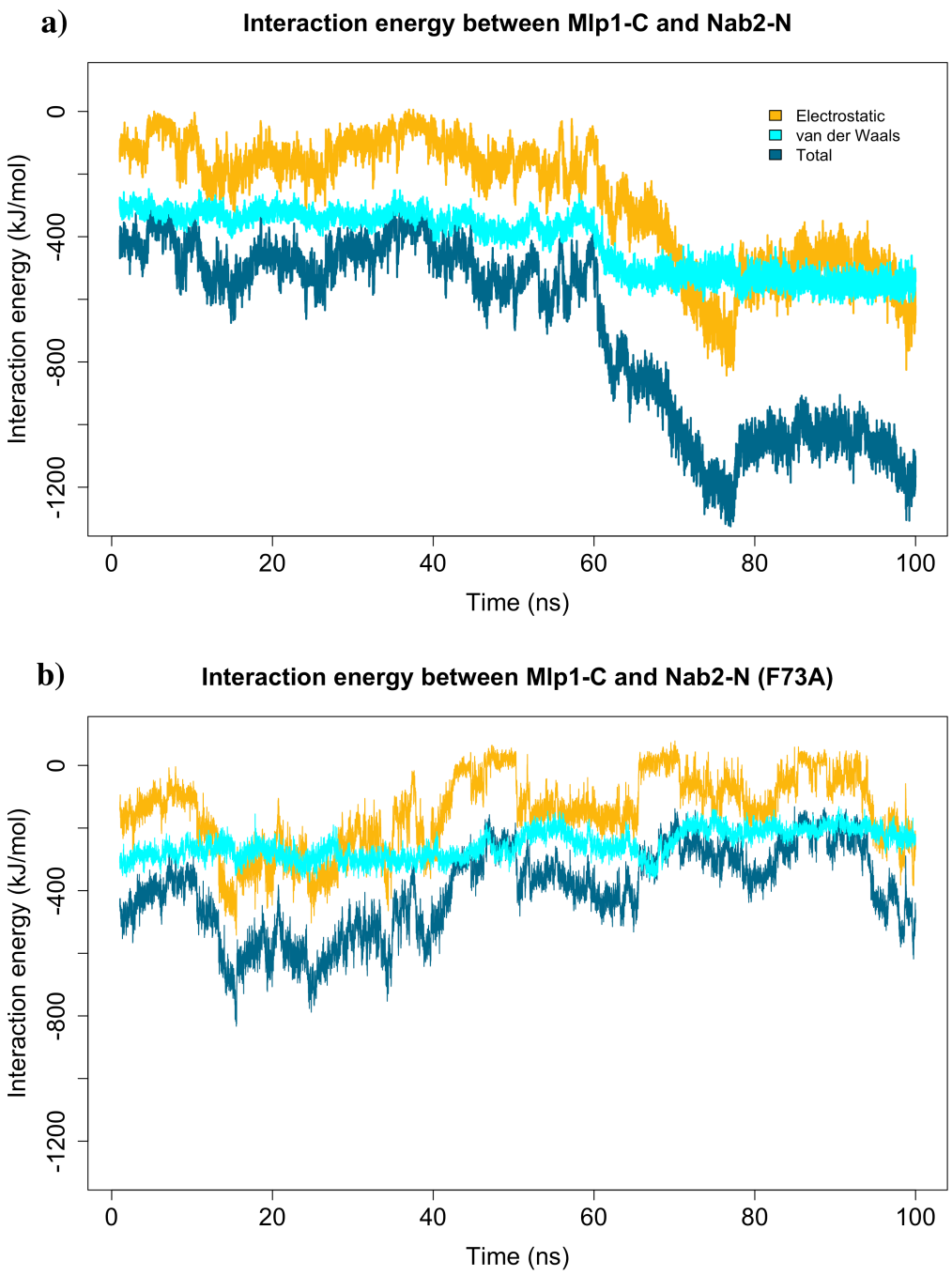

Figure 8: The interaction energies between Mlp1-C and Nab2-N during the equilibration process. a) The binding energy of the wild-type Nab2 and Mlp1 shows a three-fold increase compared to, b) that of the mutant Nab2 (Nab2-F73A).

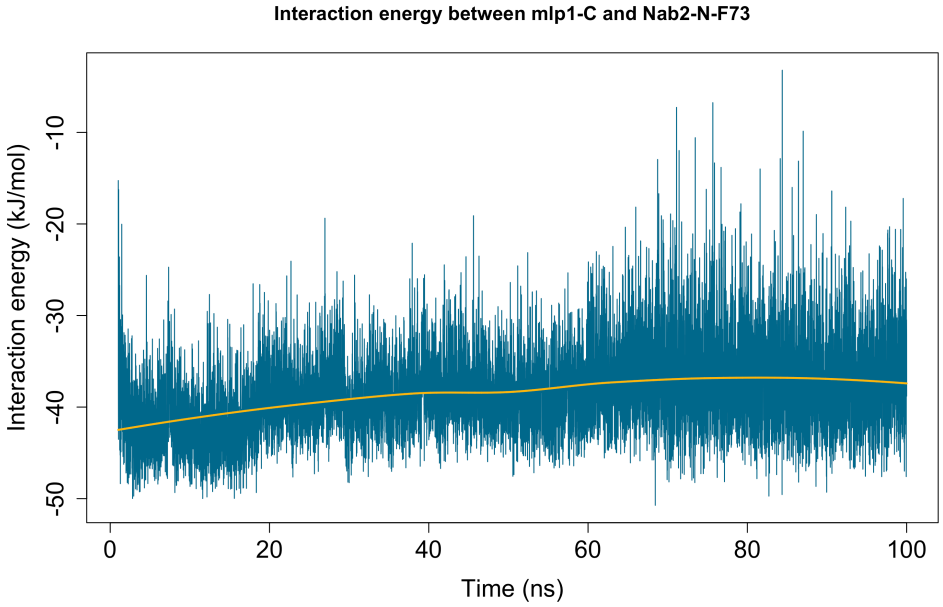

Figure 9: Interaction energy between Mlp1-C and F73 of Nab2-N. The graph shows almost constant hydrophobic interaction between the two, which implies that the increase in interaction energy between Nab2-N and Mlp1-C does not happen through direct interaction of F73 with Mlp1-C, and there are probably other mechanisms involved.

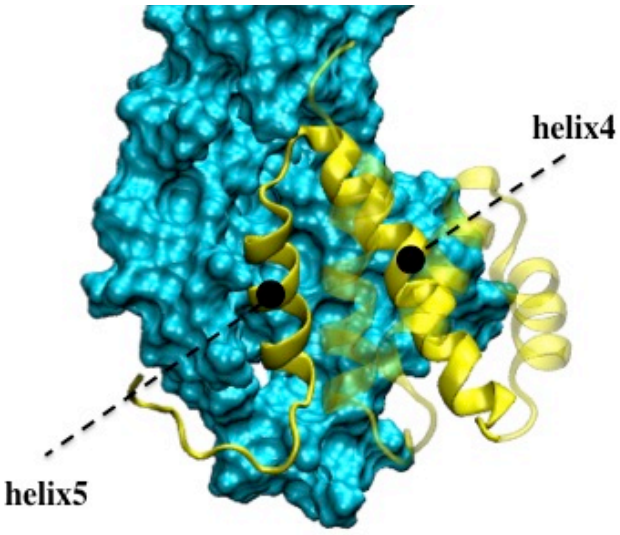

Figure 10: Nab2-N (yellow) consists of five helices, two of which are in direct contact with Mlp1-C (cyan). Nab2-F73 is located in the middle of helix4 and interacts with the hydrophobic residues of helix5.

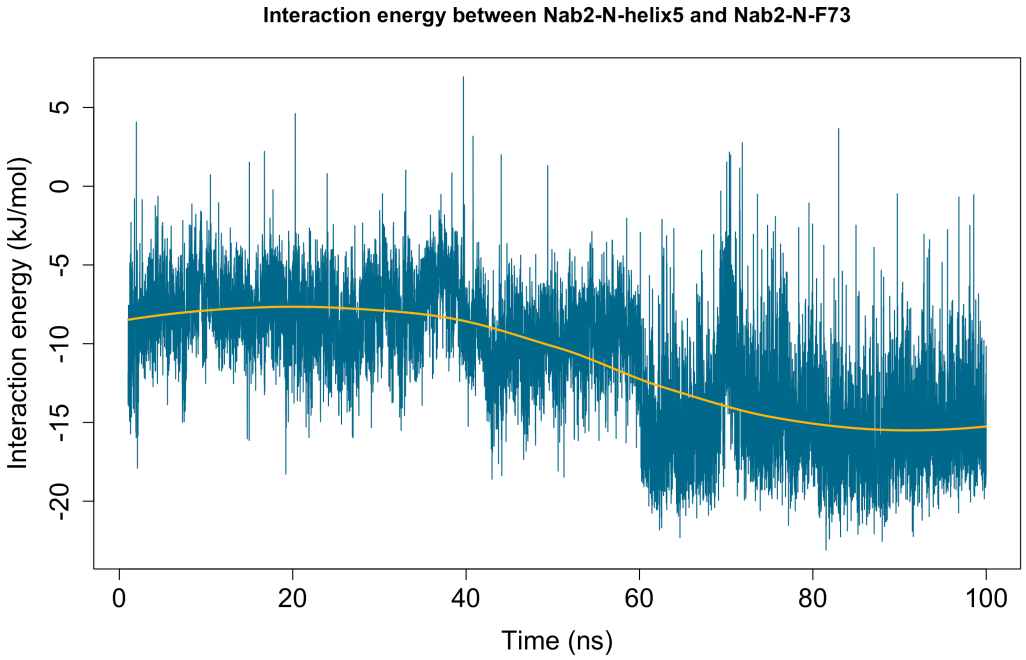

Figure 11: The interaction energy between F73 and Nab2-N-helix5. This energy plot indicates that most likely F73 affects binding between Mlp1-C and Nab2-N through its interaction with helix 2 of Nab2-N.

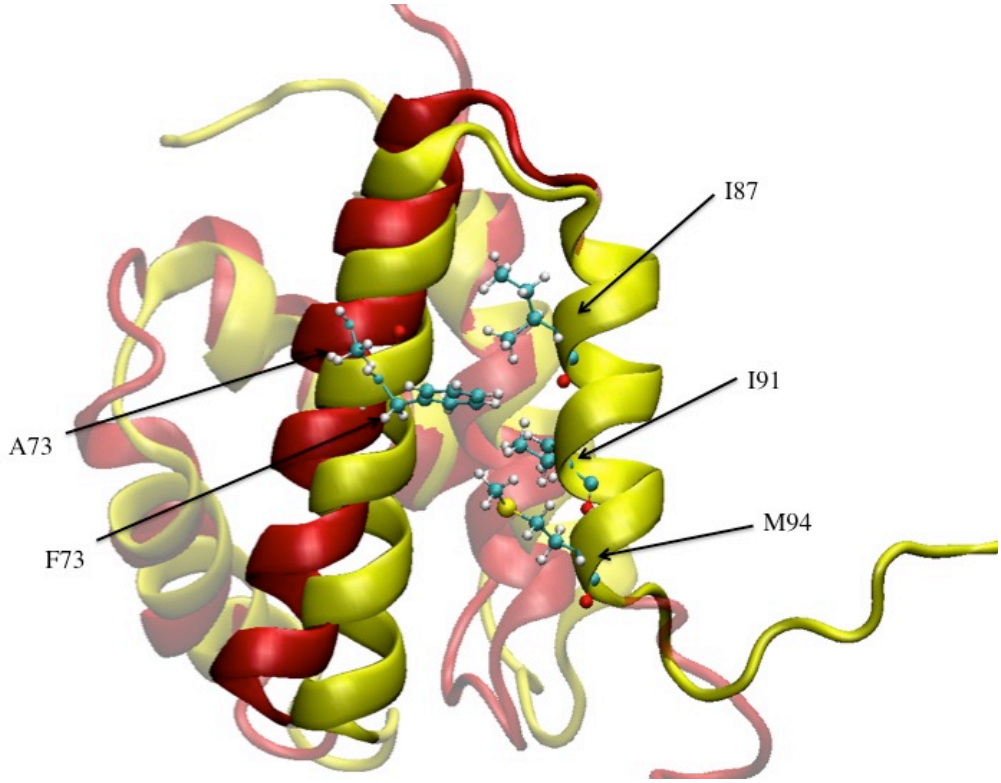

Figure 12: The interaction between the hydrophobic residues of helix4 and helix5 of Nab2-N is essential for the interaction between Mlp1-C and Nab2-N. The wild-type Nab2 is shown in yellow, while mutant (F73A) is shown in red. Nab2-N-F73 interacts with three hydrophobic residues on helix5. F73A mutation disrupts this interaction and increases the distance between the two helices.

Taken together, these observations suggested that F73 enables both helix4 and helix5 to strongly engage with Mlp1-C. At the point where the increase in the interaction energies is seen (60 ns), the benzyl side chain of F73 is locked between three hydrophobic residues from helix5 and pulls the two helices toward each other, which results in a decrease in the distance mentioned previously. Therefore, the reason behind dysregulation of the interaction upon F73A mutation could be explained by considering the fact that phenylalanine has a big benzyl side chain, enabling it to reach the hydrophobic residues on the other helix. On the other hand, alanine is a small amino acid; therefore, although being hydrophobic, it cannot reach out to the hydrophobic residues on helix5 and interact with them properly. This hypothesis is in agreement with the experimental observation that F73W mutation not only preserves the strong interaction between Mlp1 and Nab2 but also increases the affinity by a factor of 1.5, as tryptophan also possesses a large benzyl side chain (Fasken et al. 2008). Our analysis of the F73W mutation also demonstrated that this mutation maintains and strengthens the interaction between the two Nab2 helices, i.e. helix4 and helix5, as well as Mlp1-Nab2 interaction. On the other hand, mutating F73 to a non-hydrophobic residue significantly weakens the interaction (Fasken et al. 2008). Figure 3 illustrates the hydrophobic interaction that takes place between the two helices and the predicted mechanism by which F73 facilitates the interaction.

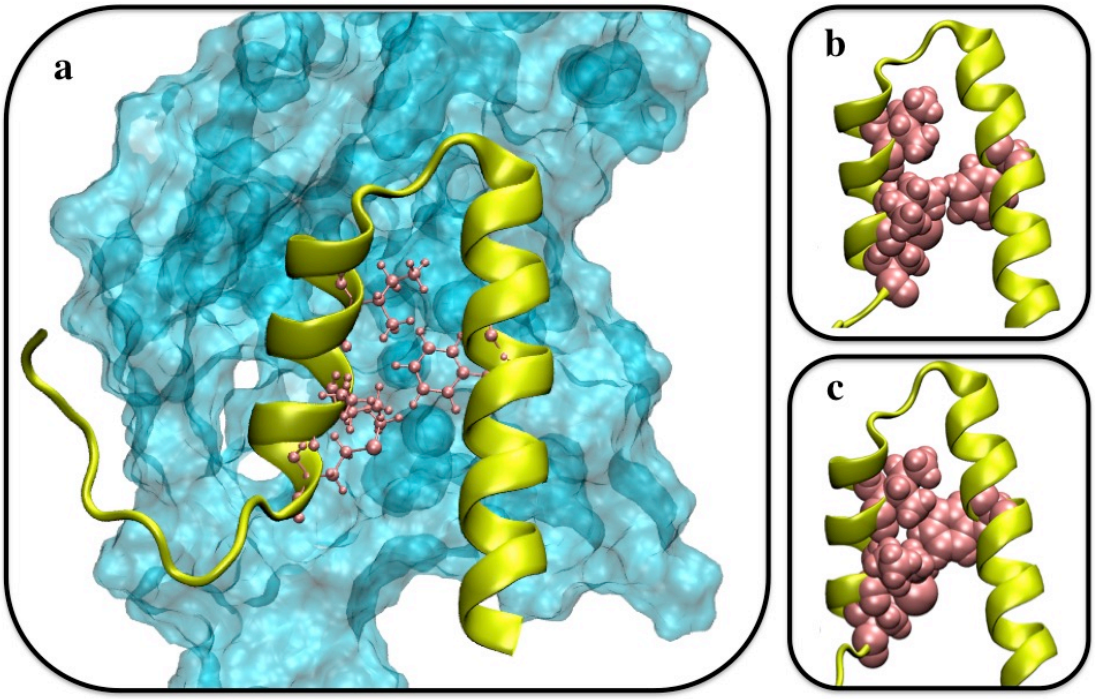

Figure 13: Role of F73 in the interaction between helix4 and helix5. Mlp1-C is shown in surface representation (transparent cyan) and Nab2-N is shown in yellow with key residues shown in pink. Please note that only the two helices of Nab2-N that are interacting with Mlp1-C are displayed. The position of the two helices of Nab2-N b) before and c) after forming a strong interaction with Mlp1-C.

In order to further validate the proposed mechanism of interaction, we mutated the three hydrophobic residues on helix5, i.e. I87, I91, and M94, to alanine. The complex of Mlp1-

C and mutated Nab2-N was equilibrated for 100 ns and subsequently simulated for an extra 20 ns, which was repeated three times to account for the stochasticity in the system. The complex energy is reduced upon the triple mutation (Figure 14), which further confirms our hypothesis on the role of F73 and the hydrophobic residues on helix5 in facilitating the Mlp1-Nab2 interaction. However, as expected, the effect of mutating helix 5 residues is not as pronounced as F73A, since the latter removes a large benzyl group critical for the binding. Furthermore, the average distance between helix4 and helix5 is measured to be 13.81 ± 0.58 Å, which is closer to the distance after F73A mutation compared to the wild-type.

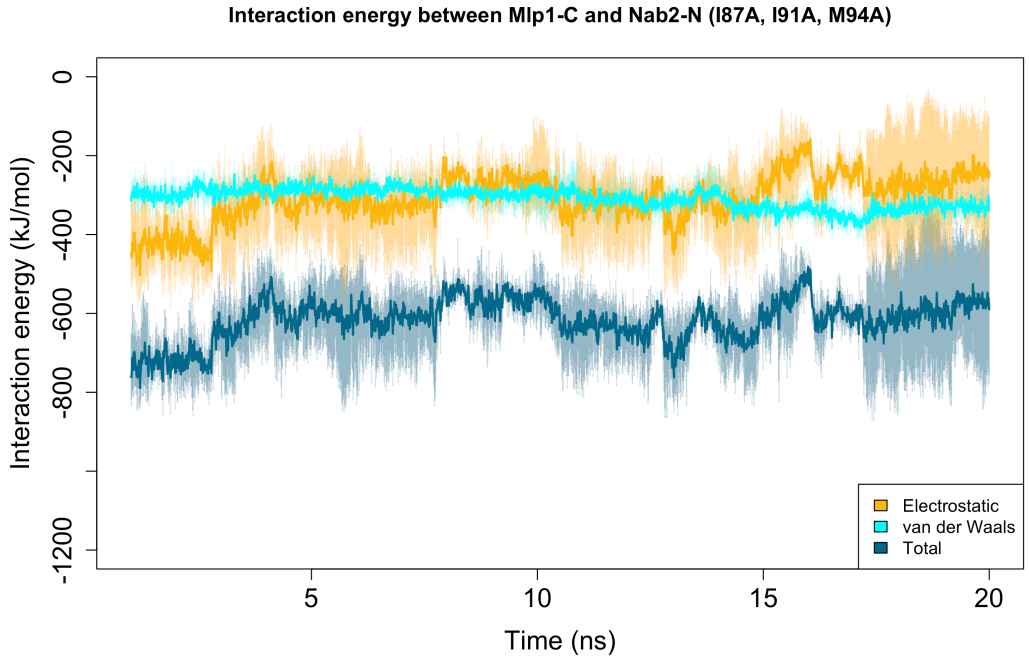

Figure 14: The interaction energy between Mlp1-C and mutated residues on helix5 of Nab2-N namely I87A, I91A, and M94A.

Discussion

Mlp1 plays a pivotal role in the quality control mechanism of mRNAs at the entry of the nuclear basket (Green et al. 2003; Fasken et al. 2008; Hackmann et al. 2014). Our results suggest that residue F73 on Nab2 plays a substantial role in the interaction between Mlp1 and Nab2 (Figure 7 and Figure 8), while F72 does not have a major effect (Figure 15), which is in agreement with previous experimental studies (Green et al. 2003; Fasken et al. 2008). Further, we showed that F73 plays an indirect role in this interaction: While the binding energy between Nab2-F73 and Mlp1-C did not change during the course of the simulation, our results showed that the interaction energy between F73 and helix5 of Nab2 slightly increased, which reduced the distance between helix4 and helix5 and resulted in a more compact conformation of Nab2-N. Consequently, the unordered domain of Mlp1-C is able to encircle Nab2-N, leading to a stronger interaction. Therefore, we suggest the following mechanism for the binding between Mlp1-C and

Nab2-N during mRNA export and quality control: 1) In the vicinity of Mlp1-C, Nab2-F73 reaches toward helix5 of Nab2 (Figure 16-a). It is important to note that, in the absence of Mlp1, it is more favorable for Nab2-F73 to move away from helix5 as shown by our simulations. We isolated Nab2 from the middle frame of the binding simulation between Mlp1-C and Nab2-N to examine how F73 behaves in the absence of Mlp1. At the beginning of the 1 ns simulation, F73 was engaged with the hydrophobic residues of helix5 (Figure 17-a). However, after about 0.1 ns, F73 rotated and pointed outward (Figure 17-b). This indicates that, the strong engagement between F73 and helix5 only happens in the vicinity of Mlp1 and is removed in isolation. 2) Nab2-F73 interacts with three hydrophobic residues on helix5 (I87, I91, and M94), which decreases the distance between the two helices (Figure 16-b); 3) Mlp1-C encircles Nab2-N and forms stronger hydrophobic interactions (Figure 16-c); 4) Following the increase in hydrophobic interactions, Mlp1-C is able to encircle Nab2-N and form significant electrostatic interactions (Figure 8 and Figure 16-d).

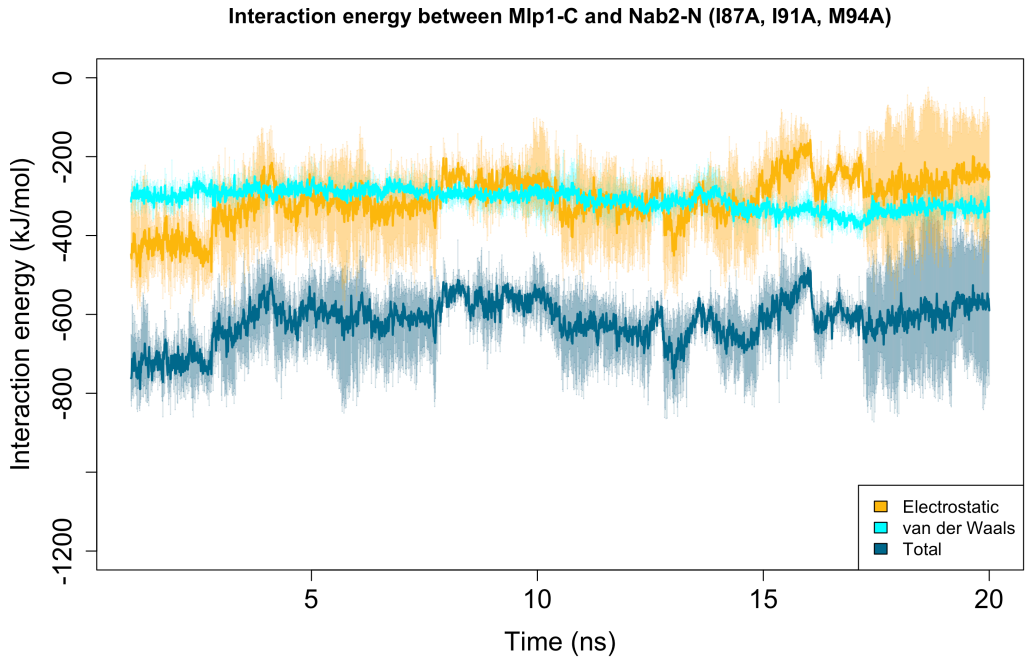

Figure 15: The interaction energy between Mlp1-C and mutated residues on helix5 of Nab2-N namely I87A, I91A, and M94A.

The electrostatic interaction formed between the coil at the end of Nab2-N and Mlp1-C in the last step of the proposed interaction mechanism (Figure 16) should be taken with a grain of salt. In this study, only the N-terminal domain of Nab2 (residues 1-97) was used, thus it could potentially be the case that inclusion of the rest of the Nab2 structure alters the interaction. However, the RGG domain of Nab2 (the domain next to the Nab2-N) is mostly disordered, allowing the coil at the end of Nab2-N to move freely. Nonetheless, other binding partners of Nab2, e.g. mRNA and export receptor heterodimer, might also affect Nab2's degrees of freedom. It is worth mentioning that the mutant complex did not

show any stable electrostatic interaction between the coil at the end of Nab2-N and Mlp1-C (Figure 8).

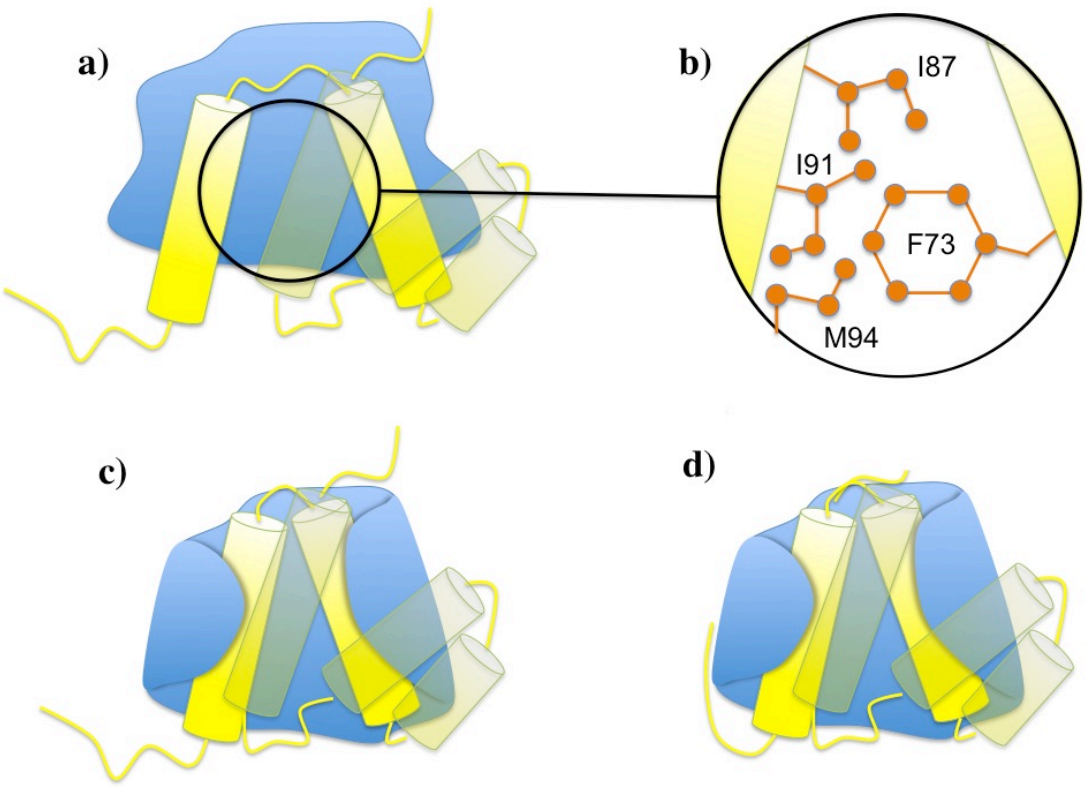

Figure 16: Schematics depicting our proposed mechanism of interaction between Nab2-N and Mlp1-C. a) Nab2-N approaches Mlp1-C as mRNA starts to export through the NPC. b) Nab2-F73 forms interactions with the three hydrophobic residues on the other helix of Nab2-N (two isoleucine and one methionine. c) As a result, the distance between the two helixes of Nab2-N that are in direct contact with Mlp1-C was decreased and the unordered region of Mlp1-C encircled Nab2-N. d) Subsequently, the coil at the end of Nab2-N was able to move around Mlp1-C and form electrostatic interactions with Mlp1-C. Only the unordered region of Mlp1-C is shown.

The C-terminal domain of Mlp1 is suggested to act as a docking site for mRNPs through interactions with various RBPs involved in mRNA export and quality control (Tutucci & Stutz 2011; Green et al. 2003; Hackmann et al. 2014). Although speculative at this point, capability of Mlp1 to bind to a range of RBPs could be attributed to the unordered region located at its C-terminal end, as explored in this study. This hypothesis, however, requires to be examined with further experimental studies to provide a deeper understanding of the quality control mechanism of mRNAs at the nuclear basket.

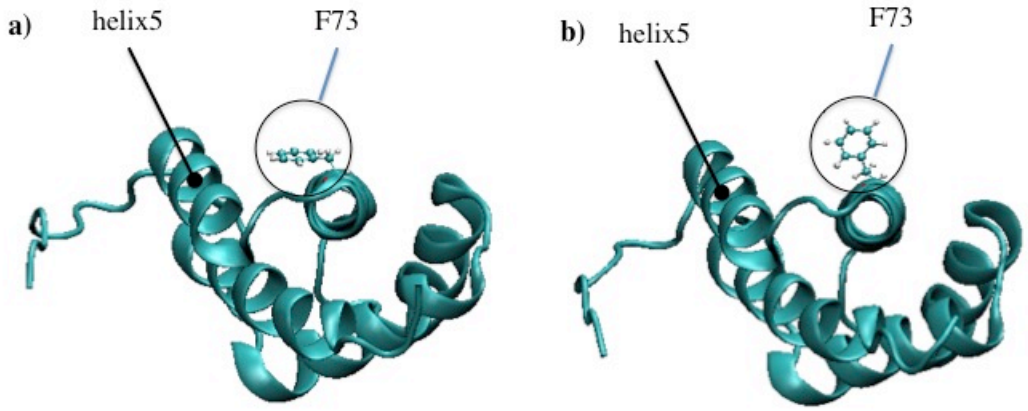

Figure 17: The presence of Mlp1-C is required for Nab2-F73 to be able to approach helix5. a) Nab2-F73 is engaged with helix5 in the bound state to Mlp1, while b) the side chain of Nab2-F73 is rotated outward once Mlp1-C is eliminated from the simulation.

Chapter 5:

Closure

Conclusions

1) **Agent-based modeling (ABM) is emerging as a widely accepted method for simulation of molecular systems:** By offering high spatial resolutions combined with long time scales as well as the unique set of capabilities, e.g. particle tracking and stochasticity, ABM provides a platform for molecular systems biology that could not be achieved through any other single computational method. In addition, integration of ABM with data-driven methods, e.g. topological data analysis (TDA), provides further capabilities in analysis of complex molecular systems.(Merelli et al. 2015) While several studies have already demonstrated the utility of ABM in molecular systems biology, ABM is not yet commonly employed in biological simulations. Specifically, some barriers have hindered ABM to become a widely used method in the biology community. Lack of ABM frameworks to reduce the complexity of model set-up has kept the community away from this powerful modeling approach. Tools such as COPASI (COmplex PAthway SImulator) have significantly simplified this process for study of biological pathways using ODEs, PDEs, or Gillespie algorithm.(Hoops et al. 2006) Regarding ABM, however, the approach for modeling these systems has been to develop the software framework from the ground up to suit the needs of the specific system being modeled. This poses a major disadvantage in that researchers spend more time on code development, validation and optimization rather than focusing on the biological problem of interest. Therefore, development of ABM frameworks specifically optimized and validated for molecular systems (similar to PhysiCell (Ghaffarizadeh et al. 2018) as a recent open-source ABM framework for multicellular systems) would substantially enhance the applicability of this powerful technique and enable a wider range of researchers to take advantage of ABM in their research.

2) Study of mRNA export and quality control requires computational approaches as well as novel high-resolution experimental techniques: Although mRNA export and quality control are widely studied to date, the dynamics of mRNA export is still elusive. One of the main challenges is the lack of experimental methods that could capture the dynamics of mRNA export with a high spatial and temporal resolution. Experimental approaches such as oligo(dT) in situ hybridization assay or single molecule fluorescence weather hybridization (smFISH) can primarily perform bulk measurements to determine the interacellular distribution of RNA but cannot capture high-resolution *in vivo* dynamics (Heinrich et al. 2017). Recent advancements in RNA labeling and imaging methods as well as novel computational techniques, however, have provided a platform to capture spatial and temporal dynamics of individual mRNAs *in vivo* (Grünwald & Robert H Singer 2010; Mor et al. 2010; Siebrasse et al. 2012; Smith et al. 2015), which enables researchers to explore mRNA export dynamics with a higher resolution both in time and in space. Computational modelling could provide high-resolution (nanometer and microsecond) details of mRNA export in long time scales (seconds), enabling researchers to evaluate the role of different factors and assess the effect of different parameters, e.g. affinities or expression levels. Novel *in vivo* methods with higher spatial and temporal resolution, such as single particle RNA-imaging (Heinrich et al. 2017), could further refine mRNA quality control hypotheses and identify the exact underlying molecular mechanisms. It is worth noting, however, that the mRNA quality control

mechanism is still under investigation and the two hypotheses discussed in this dissertation (Chapter 1) are not the only suggested mechanisms of mRNA quality control in the cell (for instance see (Porrua & Libri 2013)).

3) The potential role of the NPC in mechanotransduction is still poorly understood: It is important to realize that although the NPC is widely known for its chief role as the exclusive gateway for controlling the bidirectional traffic into and out of the nucleus; however, evidence on the interactions between the NPC components with the NE, cytoskeletal elements and the nucloskeleton suggests another essential role for the NPC as a physical linker between these important cellular components. One could therefore speculate that transport through the NPC is associated with cytoskeletal or nucleoskeletal signals, which are, in turn, partly regulated by extracellular cues. NPCs contribute to the regulation of gene expression by controlling transport of cargos, including mRNAs. This is further supported by recent evidence on the role of SUN1, which is suggested to interact with nucleoporins, in mRNA export (Li & Noegel 2015). On the other hand, NPCs are subjected to NE tensions. LINC complexes, which are embedded in the NE, are linked to both the cytoskeleton and the nucleoskeleton, and also interact with NPC components (Liu et al. 2007; Li & Noegel 2015). Therefore, either directly or indirectly through the NE or LINC complexes, NPCs are exposed to cytoskeletal and nucleoskeletal signals (Blobel 2010). Moreover, NPCs interact with elements of the nucleoskeleton and the cytoskeleton to mediate vital processes such as cytoskeletal organization, cell motility, and gene expression (Zhou & Panté 2010; Dilworth et al. 2005; Capelson et al. 2010; Chatel & Fahrenkrog 2012), further lending support to the potential role of the NPC in mechanotransduction (Mofrad & Kamm 2009). Nonetheless, few studies have examined the functional implications of NPCs as a physical linkage between the two compartments of the cell and their contribution to the regulation of nucleo-cytoskeletal coupling and mechanobiology. Further studies are required to evaluate the credibility of these hypotheses and their implications in various cellular functions.

Contributions of this dissertation

In the work presented in this dissertation, we studied the underlying mechanism of mRNA export and quality control at two levels. First, using a novel stochastic technique, we explored mRNA quality control process (Chapter 2 and Chapter 3). We showed that agent-based modelling (ABM) provides a powerful platform for researchers to explore different hypotheses of mRNA quality control. Using ABM, we demonstrated that regulation of the affinities between export receptors and RNA-binding proteins enables nuclear basket proteins to distinguish normal and aberrant mRNAs. We also showed that mRNA quality control is a length-dependent process, where it is more challenging for nuclear basket proteins to identify and retain short aberrant mRNAs.

In Chapter 4, we employed all-atom molecular dynamics (MD) simulations to show the utility of computational modelling for mRNA export and quality control at a different spatiotemporal scale. We explored the molecular interaction between two significant proteins involved in mRNA export process, namely Mlp1 and Nab2. It was previously suggested that Phe-73 of Mlp1 has a substantial role in this interaction. However, the underlying mechanism was elusive. Using molecular docking and MD, we validated the

experimentally observed role of Phe-73 in this interaction and were able to suggest a potential mechanism of interaction.

Future Directions

Agent-based modelling could be useful to explore a range of topics in molecular systems biology that are not easily tractable by current techniques. Specifically, ABM should be used in setups and systems were the continuum assumption does not hold. For example, Mofrad Lab is currently employing ABM to validate observations made using super-resolution microscopy (SRM) in the context of transport of cargos through the NPC. In this setup, nucleoproteins or constructs of them are represented by individual agents and their binding dynamics is explored. Comparing the simulation results with experimental measurements, we would be able to identify the stoichiometry of the interaction between FG Nups and transporters, which is still largely unresolved.

ABM in molecular systems biology can be further extended to incorporate other features involved in molecular pathways. Force, for example, is a key role player in many molecular processes. For instance, Reinhardt and Gooch employed ABM to model fiber networks and explored traction-force mediated matrix remodelling (Reinhardt & Gooch 2014; Reinhardt & Gooch 2018). However, force-enabled ABMs are rarely employed in molecular biology, primarily due to lack of a solid foundation to accurately represent forces in agent-based models. Therefore, this avenue of research could be further explored by the community to enable ABMs to accurately account for biological forces to be able to explore processes such as cellular adhesion at the molecular scale.

In addition, ABMs could be integrated with other computational methods across spatiotemporal scales. Many studies have integrated ABMs at larger spatiotemporal scales with other methods to explore multiscale dynamics of complex systems. For instance, cellular-scale ABMs are used for modelling of microbial biofilms and are integrated with ordinary differential equations (ODEs) for intracellular dynamics and signaling (Rudge et al. 2012). As another example at a larger spatial scale, Rozen et al. integrated an ABM, where agents are building owners, with MODFLOW/SEAWAT geohydrological modeling environment to explore the social-geological system of aquifer thermal energy storage (ATES) (Jaxa-Rozen et al. 2019). Similarly, molecular ABMs could be integrated with other methods at smaller (e.g. molecular dynamics) or larger spatiotemporal scales (e.g. cellular-scale ABM or continuum mechanics).

As for mRNA export and quality control, there are many unknowns that are still unexplored. For instance, how the distribution of RBPs on mRNA transcript affects the quality control process is not explored. The effect of other nuclear machineries, e.g. degradation, on mRNA quality control process is still under investigation. It is also still unclear whether the quality control process only selects normal mRNAs for export (selection model) or, instead, retains aberrant mRNAs inside the nucleus (retention model) (Bonnet & Palancade 2015).

Final Remarks

I started my PhD studies at UC Berkeley focusing on Computational Biology with very limited relevant background in biology or the computational techniques used to explore it. Yet, the wonderful world of biology attracted me more and more each day. At the same time, I have come to understand the challenges in exploring biological systems. Although novel experimental and computational techniques are developed each day around the globe to further complete the toolset available to study intricate biological systems, we are still largely limited in our understanding of molecular and cell biology. Biological systems are extremely intricate such that no single method could capture the full spectrum of their dynamics and properties. Experimental methods are limited in many ways such as low spatiotemporal resolution, while computational models are, oftentimes, not necessarily a complete representation of the system of interest. Therefore, It is significantly important for scientists to understand the limitations of the methods they employ when inferring the results of their studies. Given my interest in computational biology, I hope that the power of computational techniques (hand in hand with experimental techniques, of course) would be further employed in biological studies to improve our understanding of the molecular and cellular world of biology.

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