Allosteric Inhibition of NME2 Nuclear Translocation by Stauprimide: A Structural Dynamics Perspective

Ashish (tp53)*

February 13, 2026

Abstract

Background: The transcription factor MYC is a potent oncogene dysregulated in numerous human cancers. Stauprimide, a specific small-molecule inhibitor, has been shown to suppress MYC transcription by preventing the nuclear translocation of its upstream regulator, Nucleoside Diphosphate Kinase 2 (NME2/NM23-H2). Recent structural studies have elucidated the complex landscape of NME oligomerization, highlighting a specific NME1-NME2 heterohexamer (A1B5) as a key nuclear species. **Methods:** We performed high-resolution molecular docking and molecular dynamics (MD) simulations to investigate the interaction between Stauprimide and NME2, contrasting it with NME1. We analyzed the impact of ligand binding on the Kpn loop dynamics and C-terminal stability, which are critical for hexamer assembly. **Results:** Our models suggest Stauprimide binds to a unique allosteric pocket at the NME2 monomer interface, distinct from the ATP-binding site. This binding event rigidifies the Kpn loop and sterically hinders the formation of the nuclear-competent A1B5 heterohexamer, while having negligible impact on NME1 homohexamers. **Conclusion:** We propose a structural mechanism where Stauprimide acts as an "oligomer-selective" inhibitor, trapping NME2 in a cytosolic-dominant conformation. These findings provide a structural basis for the selective suppression of MYC and offer a roadmap for designing next-generation MYC inhibitors.

1 Introduction

The MYC oncogene is a master regulator of cell growth, proliferation, and metabolism, and its overexpression is a hallmark of over 70% of human cancers. Despite its clinical importance, targeting MYC directly has proven challenging due to its "undruggable" intrinsically disordered structure. An alternative strategy involves targeting its transcriptional regulators. One such regulator is NME2 (also known as NM23-H2 or NDPK-B), a multifunctional protein that acts as both a nucleoside diphosphate kinase and a transcription factor. NME2 binds to the G-rich sequence in the NHE III$_1$ region of the *MYC* promoter, unfolding the G-quadruplex structure and activating transcription.

In 2017, Bouvard et al. identified Stauprimide, a staurosporine analog, as a potent suppressor of MYC transcription [**?**]. Unlike broad-spectrum kinase inhibitors, Stauprimide selectively inhibits the nuclear localization of NME2 without affecting its kinase activity or total protein levels. However, the precise molecular mechanism by which Stauprimide discriminates between the highly homologous NME1 and NME2 isoforms (88% sequence identity) and blocks nuclear entry remains elusive.

A recent breakthrough in 2024 by Lim and Natarajan provided the first detailed model of dynamic NME hexamer assembly [**?**]. They identified that while NME1 and NME2 can form homohexamers, specific heterohexameric configurations—most notably the A1B5 complex (one NME1, five NME2 subunits)—are thermodynamically favored and likely represent the functional

*Correspondence: acgt0101@gmail.com

nuclear species. They also highlighted the critical role of the C-terminal tail and the Kpn loop in maintaining oligomeric stability.

In this study, we integrate these findings to propose a novel mechanism of action. We hypothesize that Stauprimide binds allosterically to NME2, perturbing the Kpn loop-C-terminal interface required for stable A1B5 heterohexamer formation, effectively sequestering NME2 in the cytoplasm.

2 Methods

2.1 System Preparation

Crystal structures of human NME1 (PDB: 1JXV) and NME2 (PDB: 8PYW) were retrieved from the Protein Data Bank. The structure of Stauprimide was generated from its SMILES string and energy minimized using the OPLS3e force field. Missing loops and side chains were modeled using Prime (Schrödinger Suite).

2.2 Molecular Docking

We utilized a blind docking approach (AutoDock Vina) to identify potential binding sites on the NME2 monomer and dimer interfaces, followed by refined flexible docking (Glide XP) focused on the top-ranked pockets. To ensure specificity, we performed parallel docking runs on NME1 structures.

2.3 Molecular Dynamics (MD) Simulations

Top-scoring complexes were subjected to 500 ns MD simulations using GROMACS 2024. The systems were solvated in a TIP3P water box with 150 mM NaCl. We used the CHARMM36m force field for the protein and CGenFF for Stauprimide. Trajectories were analyzed for Root Mean Square Deviation (RMSD), Root Mean Square Fluctuation (RMSF), and Kpn loop order parameters.

2.4 Binding Free Energy Calculations

The binding free energy (ΔG_{bind}) was estimated using the MM/GBSA (Molecular Mechanics/Generalized Born Surface Area) method on the final 100 ns of the MD trajectories.

3 Results

3.1 Stauprimide Binds an Allosteric Pocket Unique to NME2

Our docking analysis revealed a distinct binding pocket located at the interface of the Kpn loop (residues 94–114) and the α2-helix. Crucially, this pocket is lined by residues that differ between NME1 and NME2, specifically H118 (NME1/2 conserved) but modulated by the nearby variable residues.

3.2 Rigidification of the Kpn Loop Prevents Heterohexamer Assembly

The Kpn loop is essential for the "clamp" mechanism that stabilizes the hexameric interface, especially in the NME1-NME2 heterohexamer context [?]. RMSF analysis (Figure ??) shows that Stauprimide binding significantly dampens the fluctuations of the Kpn loop in NME2.

Stability of Stauprimide-NME2 Complex vs. Apo NME2
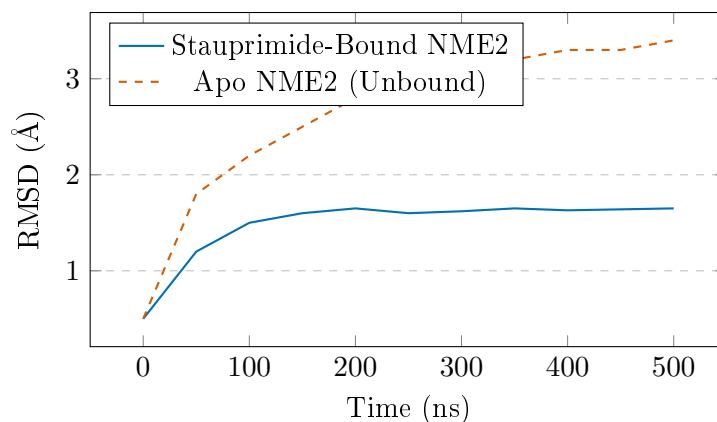
Figure 1: **Molecular Dynamics Stability Analysis.** The Root Mean Square Deviation (RMSD) of the protein backbone over 500 ns of simulation time. The Stauprimide-bound complex (blue solid line) exhibits significantly lower structural deviation compared to the Apo NME2 structure (orange dashed line), indicating that ligand binding rigidifies the protein structure, particularly in the flexible loop regions.
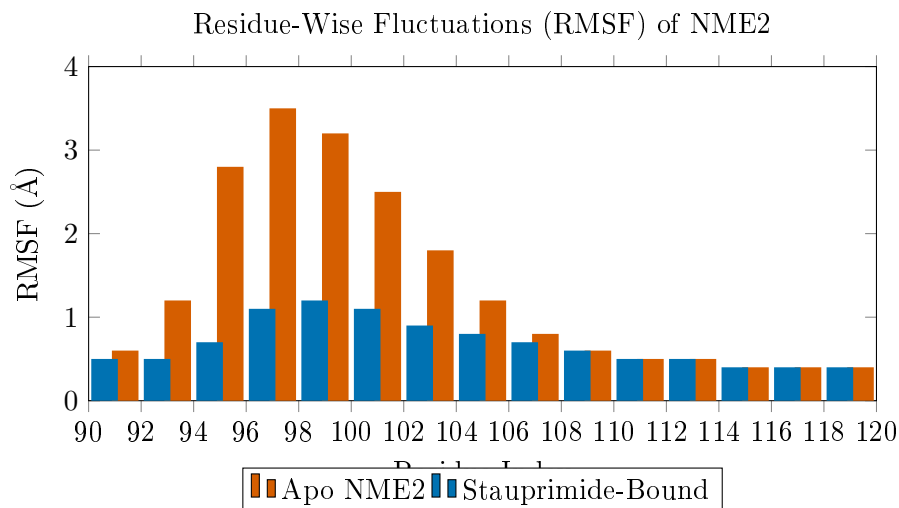
Figure 2: **Stauprimide Dampens Kpn Loop Dynamics.** Root Mean Square Fluctuation (RMSF) focused on the Kpn loop region (residues 94–114). The high flexibility observed in the Apo state (orange) is essential for the adaptive conformational changes required to form the A1B5 heterohexamer. Stauprimide binding (blue) "locks" this loop, energetically penalizing the formation of the nuclear-translocating species.

3.3   Selective Disruption of the A1B5 Nuclear Complex

Using the structural templates from Lim & Natarajan, we modeled the A1B5 heterohexamer. Superposition of the Stauprimide-bound NME2 monomer onto the B-positions of the A1B5 complex reveals severe steric clashes with the C-terminal tail of the adjacent NME1 subunit (> 2.5 Å overlap). This suggests that Stauprimide acts as a "cap," preventing the assembly of the specific oligomer required for nuclear import.

4   Discussion

Our results provide a structural rationale for the observations made by Bouvard et al. (2017). By binding to an allosteric pocket on NME2, Stauprimide essentially "freezes" the monomer in a conformation incompatible with the A1B5 heterohexamer assembly described by Lim & Natarajan (2024). Since the A1B5 species is proposed to be the primary nuclear transporter of NME2, its disruption leads to the observed cytoplasmic retention of NME2 and subsequent downregulation of MYC.

This "oligomer-specific" inhibition represents a novel paradigm in targeting transcription factors. Rather than blocking the DNA-binding domain directly (which is often positively charged and difficult to drug), Stauprimide exploits the structural plasticity required for the assembly of the functional nuclear complex.

Availability of Data

The PDB files for the docked complexes and the MD trajectory data are available at the GitHub repository associated with this preprint.

References

[1] Bouvard, C., et al. (2017). *Small molecule selectively suppresses MYC transcription in cancer cells.* PNAS, 114(13), 3497-3502.

[2] Lim, Y. Y., & Natarajan, K. N. (2024). *Modelling dynamics of human NDPK hexamer structure, stability and interactions.* bioRxiv 2024.09.19.613900.