

Individual Research Project Presentations Day 10th June 2024, Kent and Medway Medical School.

A molecular dynamic study of HIV-1 Tat protein as a potential therapeutic target.

Student^a: Grace Dabson | **Supervisor(s)^b:** Dr Kris Leslie

Abstract

Background: Research and development remain ongoing to continue improving the current landscape of HIV treatment. Important stages of the HIV life cycle such as transcription have still yet to be targeted by therapies, and one important transcriptional regulator is the ‘Trans-Activator of Transcription’, or ‘Tat’ protein. Despite widespread acknowledgement of Tat’s role in HIV replication, current information on Tat remains limited; at present the only molecular models of the protein available are of a portion of the binding site with TAR-RNA, and a model of Tat from HIV subtype B which is a less clinically relevant subtype. This project aims to produce a model of Tat subtype C both solo and docked with TAR-RNA, as well as characterising the properties, interactions, and behaviour of Tat and TAR RNA to inform future drug development.

Methods: Three molecular dynamic runs of Tat solo and two docked with RNA (one constrained, one unconstrained) were simulated using NAMD and run at Canterbury Christ Church University using accelerated GPU calculations and a 2-femtosecond integration timestep for a minimum of 500ns. VMD, PyMOL, and qtGrace software were used to run, visualise, and analyse the data produced using RMSD, hydrogen-bonding, RMSF, electrostatic mapping and PCA K-means clustering analysis.

Results: RMSD of solo Tat runs was found to be consistently higher than that of Tat when docked with RNA, both constrained and unconstrained. Hydrogen bond analysis supported this, with detailed analysis showing Arg55 – Asp67 H-bonding at 72.72% occupancy in Tat docked with constrained RNA compared to the highest occupancy of Tyr32 – Tyr26 at 24.90% for Tat solo. K-means clustering from PCA analysis again supported this, showing a large spread of data for solo Tat versus a more consistent and concentrated plot of data for Tat docked with RNA. Structural analysis allowed for visualisation, and combined with RMSF overlay showed the stretched and unstable conformation of solo Tat versus compact conformation when docked with RNA. Electrostatic mapping of these more compact structures revealed positively charged regions encompassing the core and binding functional domains, as well as a negatively charged tail which protrudes from the core structure and moves freely.

Conclusions: Solo Tat runs demonstrated the flexibility and relative unpredictability of the protein, suggesting that future drug development for Tat in unbound conformations may prove difficult. However, when docked with RNA, the more compact and stable structure may be better suited to drug design. The core and binding functional domains of Tat appear relatively strongly positively charged, and hydrogen bonding and RMSD data shows that these regions are far less subject to change. Novel models of full Tat subtype C, and Tat with TAR RNA have been produced from this project for distribution and further investigation into Tat. Repeat analysis with longer RNA strands to avoid constraining the RNA are recommended in future, as well as comparison between different HIV subtypes. Consideration of Tat interaction with other known transcription factors such as pTEFb may also prove useful, and deep docking to identify candidate drug molecules based on these findings is recommended.

Keywords: HIV-1 | Tat | molecular | dynamics

^a 4th Year Medical Student, Kent and Medway Medical School, Canterbury, United Kingdom

^b Structural Biologist, Canterbury Christ Church University, Canterbury, United Kingdom

Main contact email: g.dabson22@kmms.ac.uk