# SAMHD1'S INTERACTION WITH NUCLEOTIDE ANALOGUE MOLECULE X


## ABSTRACT

SAMHD1 is an enzyme that regulates deoxyribonucleotide triphosphate (dNTP) concentrations by hydrolysing them, thereby playing a role in antiviral defence. SAMHD1's catalytic activity depends on its tetrameric form.

This study investigates whether Molecule X, an analogue of dNTPs, can promote SAMHD1 tetramerisation and, consequently, be hydrolysed by SAMHD1.

Using size exclusion chromatography (SEC), SAMHD1's oligomeric states were analysed in the presence of GTP, dATP, and Molecule X. While GTP and dATP together facilitate the formation of the active tetrameric form of SAMHD1, GTP combined with Molecule X does not. This suggests that Molecule X does not promote tetramerisation.


## INTRODUCTION

SAMHD1 is an enzyme that specifically hydrolyses deoxyribonucleotide triphosphates (dNTPs), the building blocks of DNA. This enzyme plays a crucial role in regulating cellular dNTP levels. Molecule X is an analogue of dNTPs and is a chain terminator. Therefore, as SAMHD1 and molecule X are both involved in antiviral mechanisms, it would make sense that they have evolved to exclude one another. This study investigated whether Molecule X interacts with SAMHD1 similarly to dNTPs, using size exclusion chromatography to assess the enzyme's oligomeric states in the presence of different nucleotides.

## METHODOLOGY

SAMHD1 was expressed and purified then SEC chromatography was used to investigate its oligomeric state in the presence of different nucleotide combinations.

## RESULTS

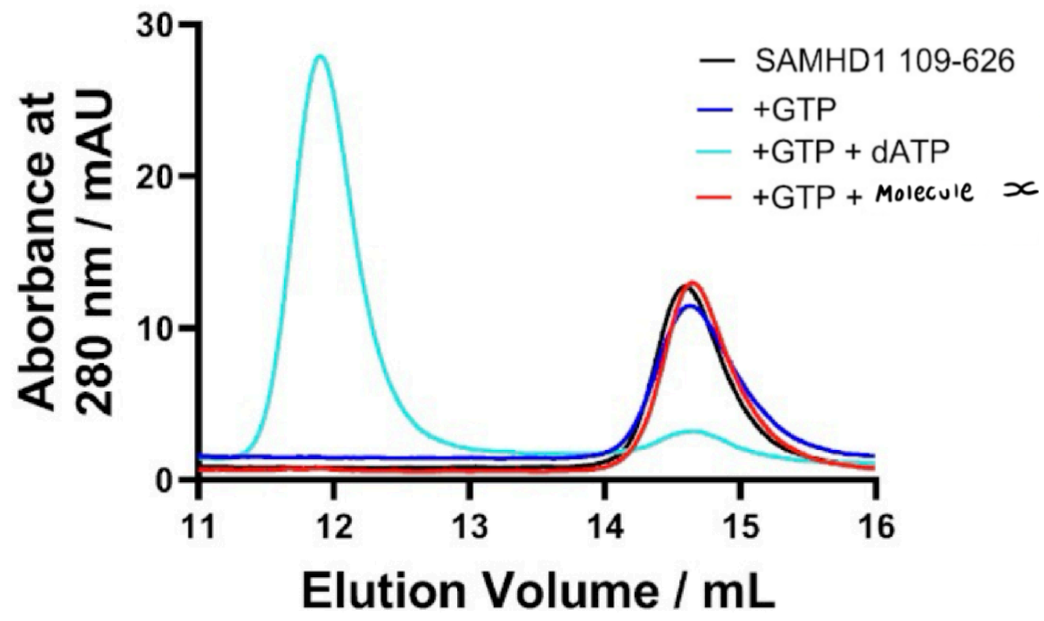


**Figure 1: Size Exclusion Chromatography elution profile of SAMHD1's catalytic domain (residues 109-626) in the Presence of Different Nucleotides.**

- SAMHD1 Alone (Black Trace): Elutes primarily as a dimer at an elution volume of ~14.5 mL.
- GTP Alone (Dark Blue Trace): Minimal shift in the elution profile suggests no significant interaction or structural change, indicating GTP alone does not stabilize the tetramer.
- GTP and dATP (Cyan Trace): A prominent peak at ~12 mL indicates tetramerisation of SAMHD1, showing that both GTP and dATP are required for forming the active tetrameric state.
- GTP and Molecule X (Red Trace): SAMHD1 remains in the dimeric form (~14.5 mL), with no peak at ~12 mL. This suggests that Molecule X does not promote tetramerisation.

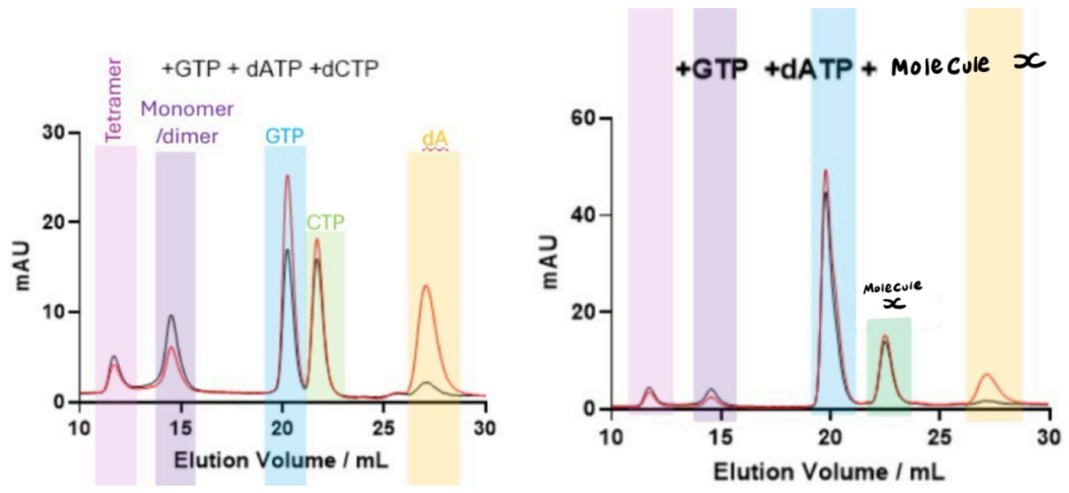


**Figure 2: Size exclusion chromatograms showing the elution of SAMHD1 and nucleotides in the presence of different nucleotide combinations**

Left Panel: SAMHD1 incubated with GTP, dATP, and dCTP displays a prominent tetramer peak at ~12-14 mL, indicating successful tetramerisation. Peaks corresponding to monomers/dimers appear at ~15-18 mL, and free nucleotides (GTP, dCTP, dATP) elute at ~20-25 mL. Right Panel: SAMHD1 incubated with GTP, dATP, and Molecule X shows a reduced tetramer peak, suggesting Molecule X is less effective in promoting tetramerisation. A distinct peak for free Molecule X at ~20 mL indicates it does not bind effectively to SAMHD1. This suggests that Molecule X, despite being an analogue of natural dNTPs, does not interact with SAMHD1 to stabilize its active tetrameric form

## CONCLUSION

**Molecule X does not induce tetramerisation of SAMHD1.**

Further research should focus on confirming whether SAMHD1 hydrolyses molecule X using enzymatic assays and investigating the interaction kinetics to determine the chemical reasons for the lack of tetramerisation. Understanding this dynamic could reveal how SAMHD1 discriminates between its natural substrates and analogues, potentially informing the development of antiviral strategies utilising this mechanism.

## AUTHORS AND FUNDING


Researcher: Emily Jack (Biosceinces Undergraduate Durham University)
Supervisor: Liz Morris (Assistant Professor Durham University)
PHD supervisor: Rosella Pickney (Research Postgraduate Durham University)


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FIGURES PREPARED BY ROSELLA PICKNEY