

OPTIMIZATION AND VALIDATION OF A BIOCHEMICAL FRET ASSAY FOR THE EXONUCLEASE ACTIVITY OF SARS COV-2 NSP14-NSP10 COMPLEX

Laura Dettori^{1,2}, Paolo Malune², Marta Maria Cara^{1,2}, Salvatore Nieddu², Enzo Tramontano² and Francesca Esposito²

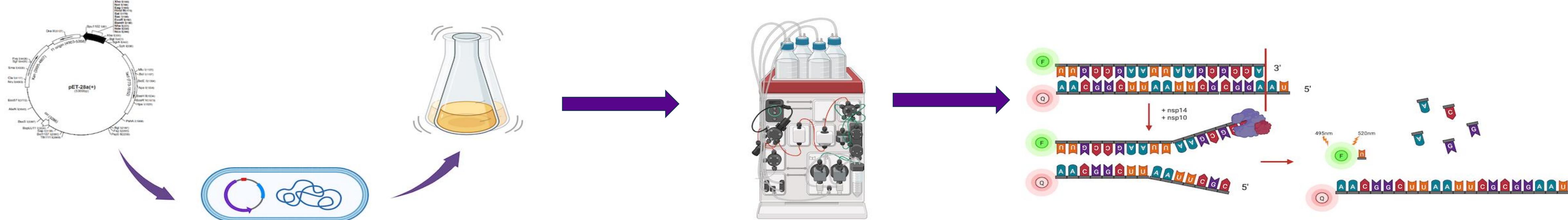
¹National PhD Programme in One Health approaches to infectious diseases and life science research, Department of Public Health, Experimental and Forensic Medicine, University of Pavia, Pavia, 27100, Italy.

²Department of Life and Environmental Sciences, University of Cagliari, 09042, Monserrato, Italy.

INTRODUCTION

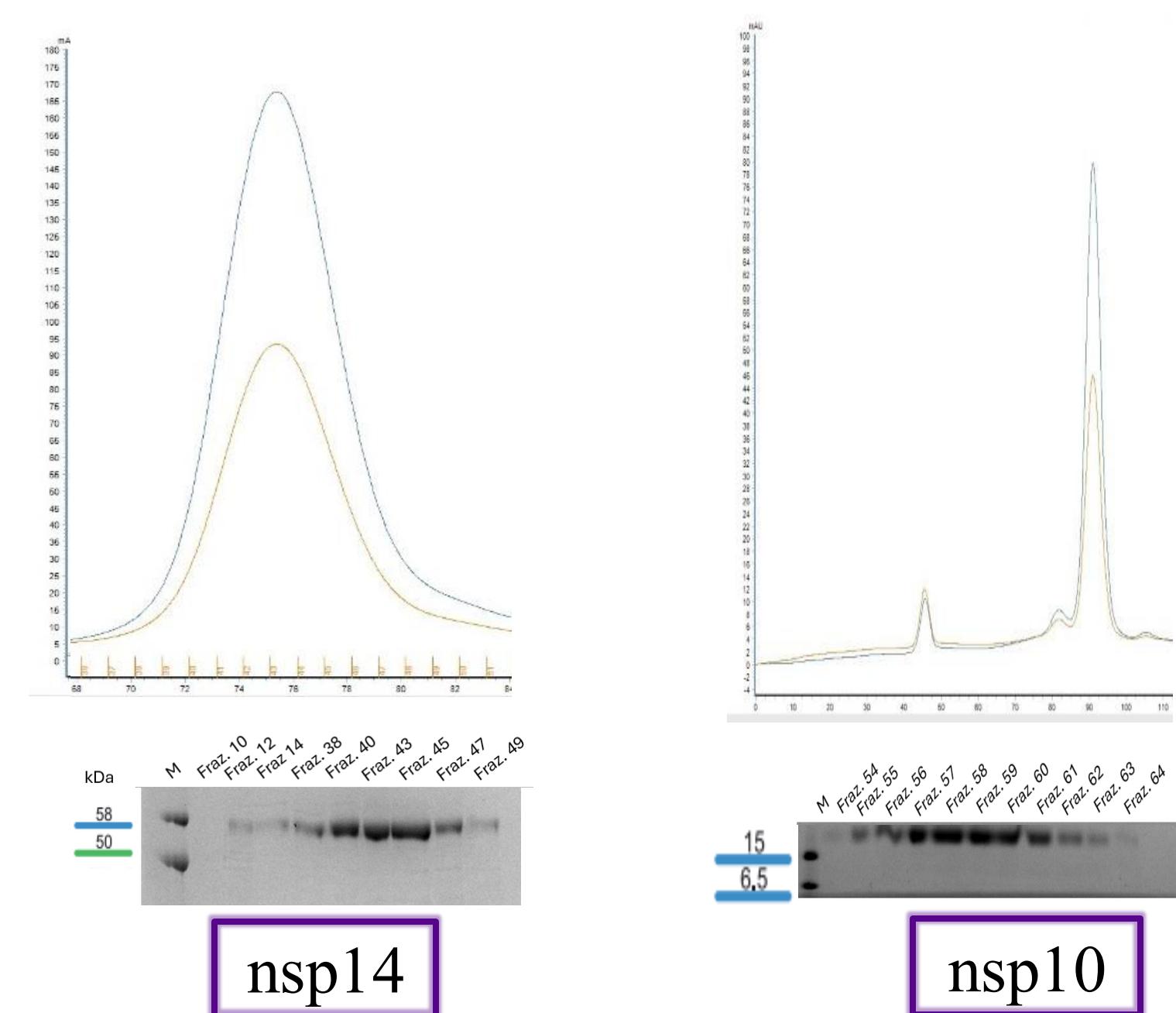
SARS-CoV-2, the virus responsible for COVID-19, has one of the longest RNA genomes among RNA viruses. To correct replication errors, it uses a unique proofreading mechanism involving the nsp14 protein, which has 3'-5' exonuclease activity, assisted by the cofactor nsp10. Currently, there are no drugs targeting this function. To support antiviral research, nsp14 and nsp10 were expressed and purified in E. Coli cells, and a FRET-based biochemical assay was developed to study their exonuclease activity, as previously mentioned in the literature [1]. This system lays the groundwork for identifying future inhibitors of the nsp14-nsp10 complex.

WORKFLOW

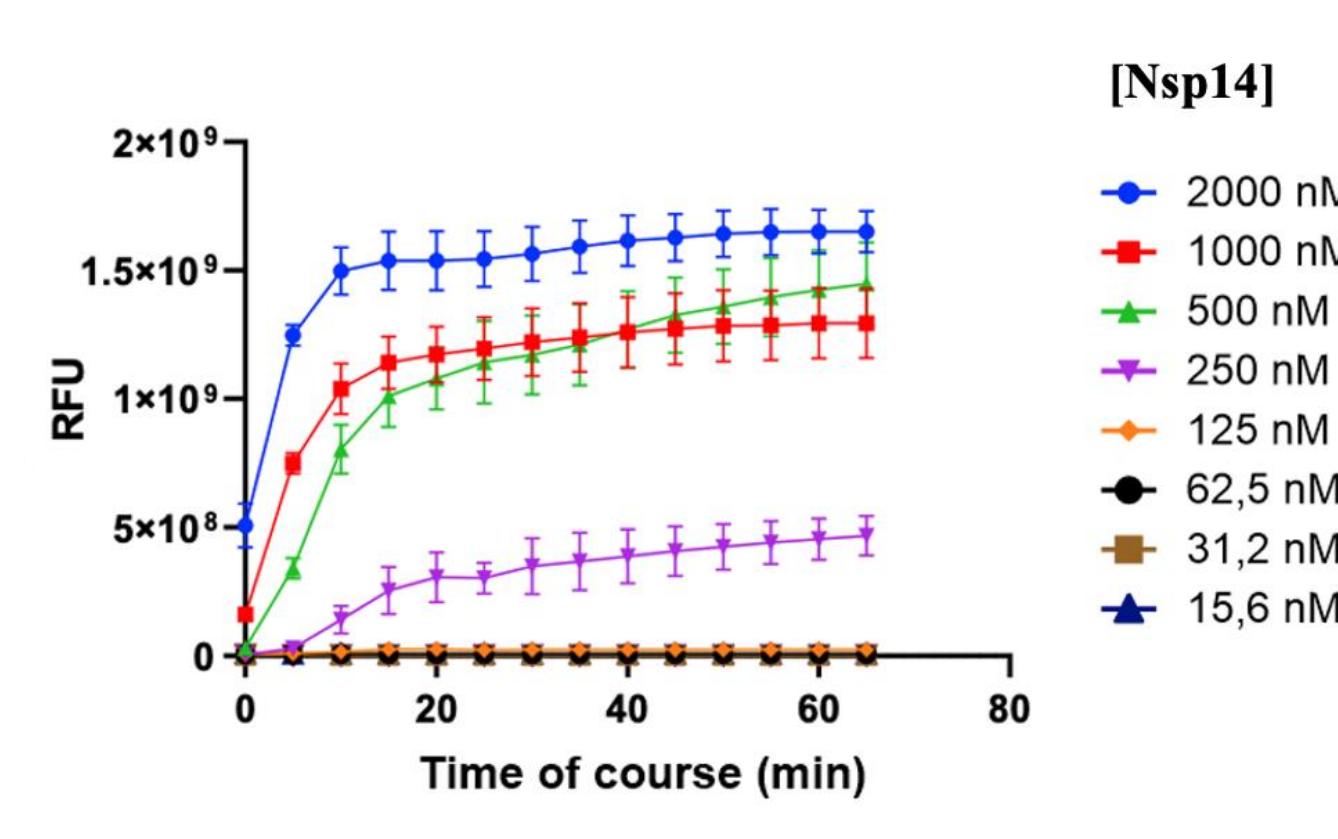


RESULTS

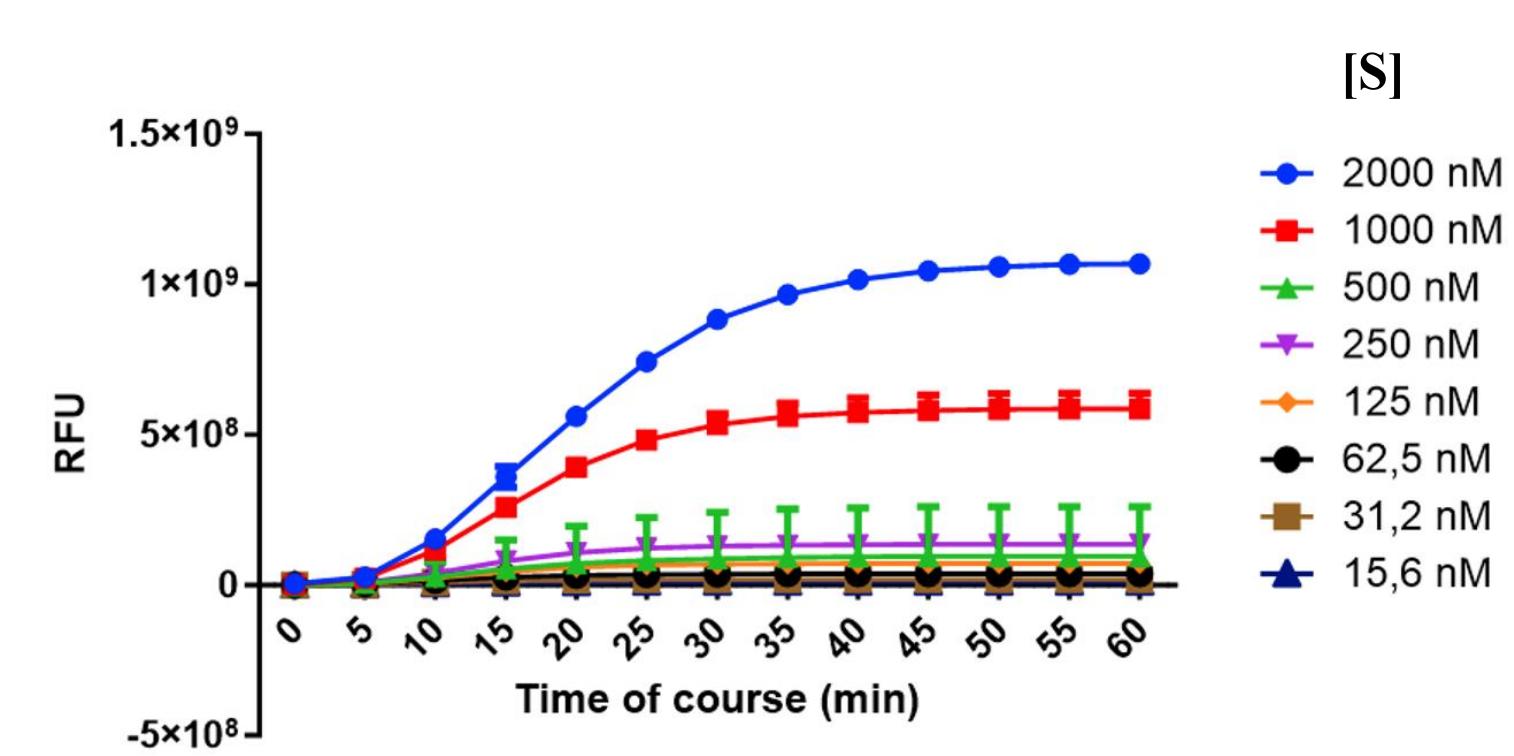
1. Size-exclusion chromatography (SEC)



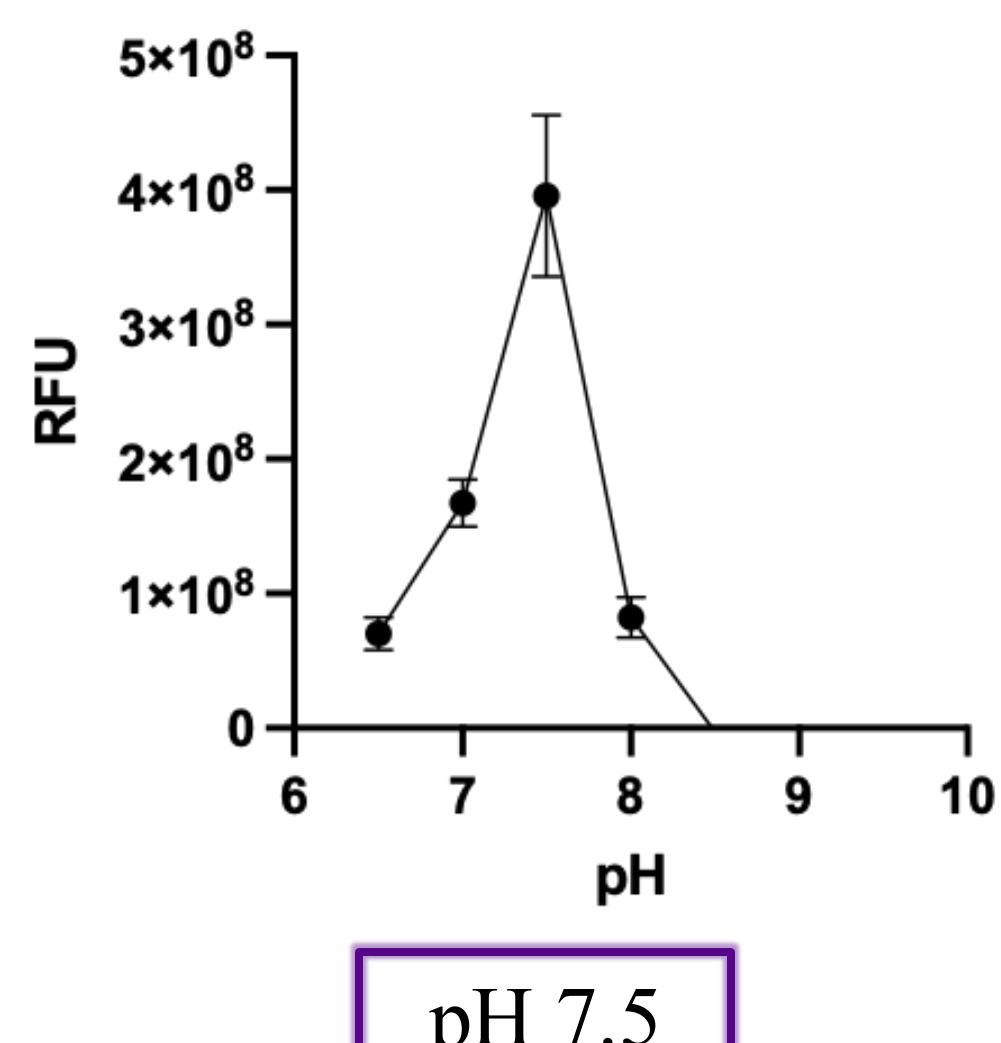
4. Enzymatic activity curve



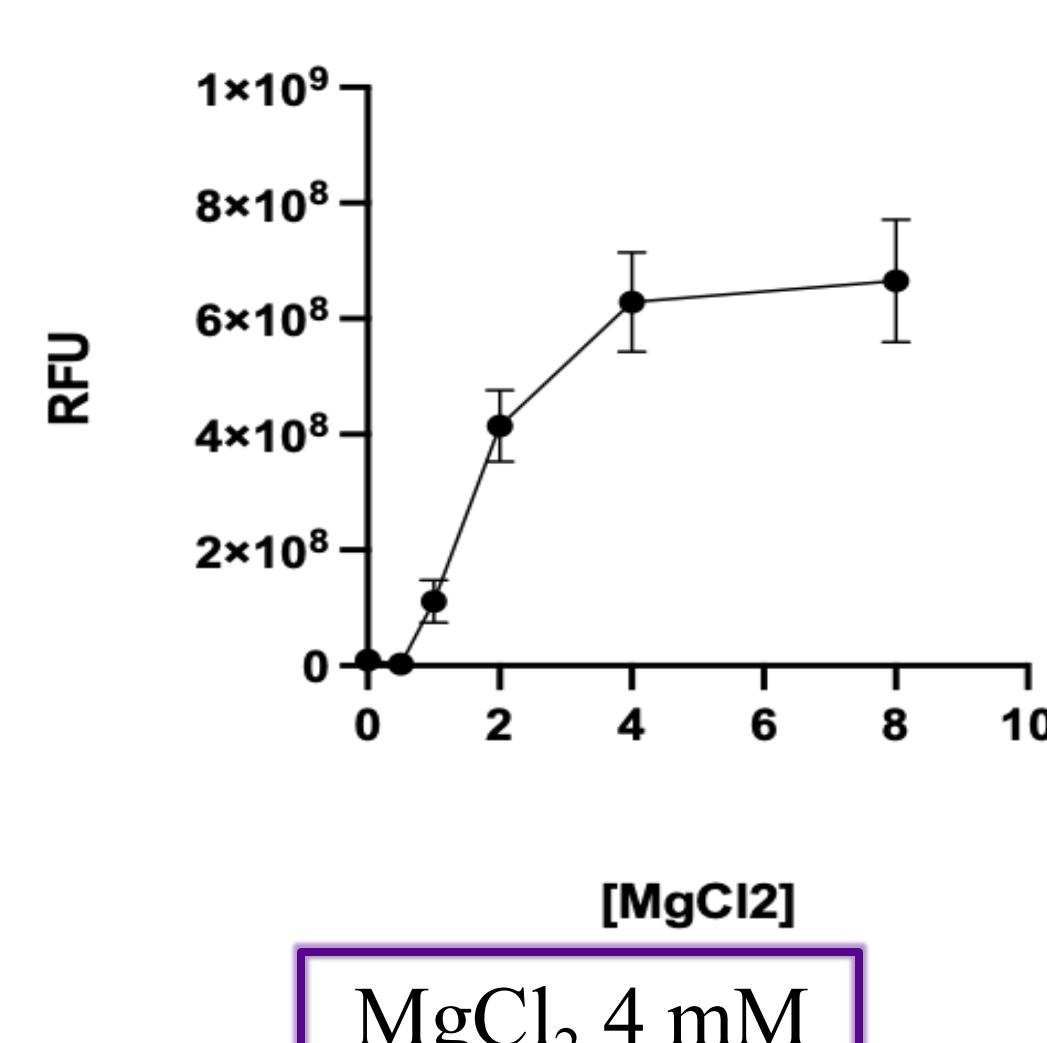
5. Substrate concentration curve



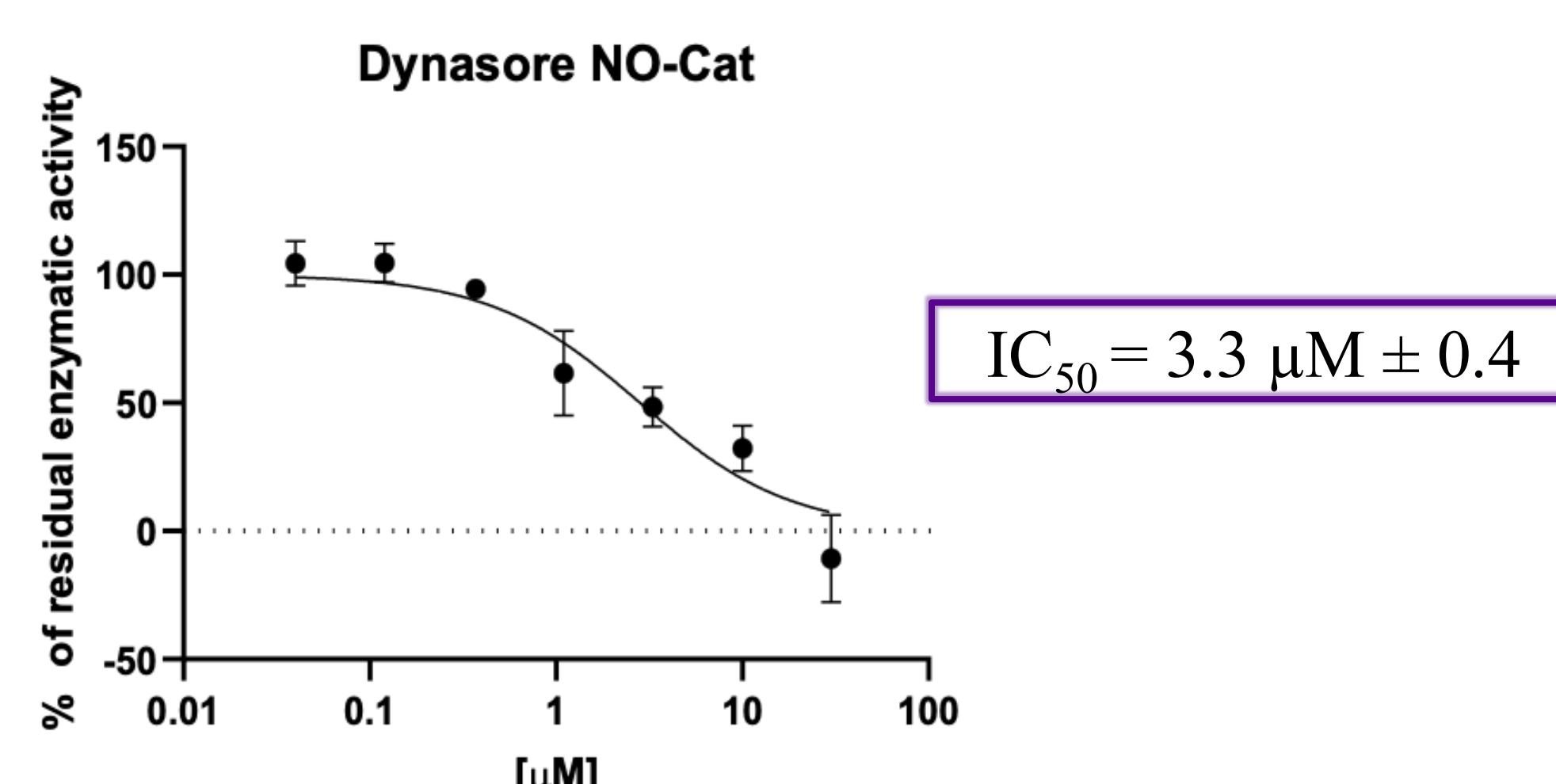
2. pH dose-dependent



3. MgCl₂ dose-dependent



6. Inhibition Dynasore NO-Cat curve



CONCLUSIONS

Optimization of the biochemical assay of the enzyme nsp14-10 allowed the development of a sensitive, reproducible, and effective method to study the activity of this enzyme complex. A compound known in the literature as an inhibitor of the nsp14-10 complex was used [2]. This result represents an important step in furthering the biochemical characterization of nsp14-10 and facilitating future screening activities for potential inhibitors, with possible implications in the development of new antiviral strategies.

ACKNOWLEDGMENTS

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[1] Rona G, Zeke A et al. The NSP14/NSP10 RNA repair complex as a Pan-coronavirus therapeutic target. *Cell Death Differ.* 2022 Feb;29(2):285-292. [2] Asthana, A.; Corona, A.; et al. Analogs of the Catechol Derivative Dynasore Inhibit HIV-1 Ribonuclease H, SARS-CoV-2 nsp14 Exoribonuclease, and Virus Replication. *Viruses* 2023, 15, 1539.

REFERENCES